TADALAFIL DRUG FOR ERECTILE DYSFUNCTION: A REVIEW OF ANALYTICAL TECHNIQUES & CLINICAL STUDIES

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ABSTRACT: Erectile dysfunction (impotency) is a type of disability in sexual behavior of male in which penis erection is difficult or none to develop during copulation. This condition is very common nowadays, more than half of men ages 40-70 are affected by this. Treatment of erectile dysfunction (ED) is depends on its cause, some of the patient only needs an aerobic exerecise while people who used to drink or smoke, cessation, gives best result to them and most of the people need medication for the treatment. The present review describes information regarding an erectile dysfunction drug- Tadalafil, which is approved for the treatment of impotency, prostatic hyperplasia & pulmonary hypertension. The present review includes all available information like pharmacokinetics, pharmacological action & side effects. It also provides information regarding various analytical methods developed for this drug along with its validation parameters and different clinical aspects of the drug.

Keywords: Erectile dysfunction, Tadalafil.

I.   INTRODUCTION:

Erectile dysfunction(ED), also known by male impotency [1, 2]. It is a type of inability in sexual behavior of male in which penis erection is difficult or none to develop during copulation. It is not a disease but a sign that some others problem are there. Nowadays, ED is very common condition, more than half of men ages 40-70 are affected by this. ED caused by different reasons, it involves diabetes, cardiovascular disease, smoking, alcohol, kidney failure, nervous system disorders, psychological disorders(anxiety, depression) and due to side effects of some medications. Treatment of ED is totally depend on cause, like some need only exercise while people who consume alcohol or have smoking habit need to quit

this. Most of the people need medication for the treatment; Tadalafil is one of the main effective medicines for ED [2,3,14].

Tadalafil is an agent to treat impotency, prostatic hyperplasia & pulmonary hypertension[2]. But above all this it is mainly used in the treatment of erectile dysfunctioning. It is an oral dosage form marketed in form of a pill. Chemically, it is indicated as pyrazino-[1',2':1,6]pyrido[3,4-b]indole-1,4-dione,6-(1,3-benzodioxol-5-yl)-2,3,6,7,12,12a-hexahydro-2-methyl-,(6R,12aR). It is a selective phosphodiesterase type5 (PDE5) inhibitor which prevent cyclic guanosine monophosphate (cGMP) from being chemically deteriorate in penile tissue [1,3,4,5,13].

Tadalafil is approved by USFDA in 2003 to treat erectile dysfunctioning & in October 2011 approved for prostatic hyperplasia. Officially, it is not proclaimed in any Pharmacopoeia. Therefore further research on this oral drug is requiring. But it is listed in Martindale which is Complete Drug Reference and in Merck Index [1,4].

II. DRUG PROFILE:

Synonyms:

- (6R,12AR)-2,3,6,7,12,12a-hexahydro-2-methyl-6-[3,4-(methylenedioxy)phenyl] pyrazino (1',2':1,6)pyrido(3,4-b)indole-1,4-dione.
- (6R-trans)-6-(1,3-Benzodioxol-5-yl)-2,3,6,7,12,12a-hexahydro-2-methyl-pyrazino (1',2':1,6)pyrido(3,4-b)indole-1,4-dione.
- 6-BENZO[1,3]dioxol-5-yl-2-methyl-2,3,6,7,12,12a-hexahydro-pyrazino[1',2':1,6]pyrido [3,4-b]indole-1,4-dione.[13]

Molecular Formula: $C_{22}H_{19}N_3O_4$[2,3]

Molecular Mass: 389.404 g/mol

Route of Administration: Orally

Structure:
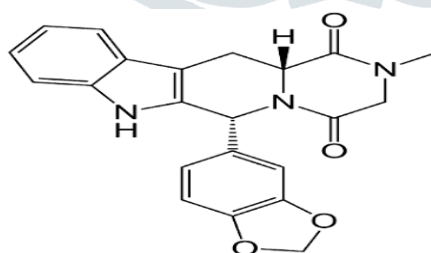
Boiling Point: 679.1 ± 55°C at 760 mmHg

Melting Point: 301-302°C

Appearance: White crystalline solid

Solubility: Soluble in organic solvent & sparingly soluble in aqueous buffer.
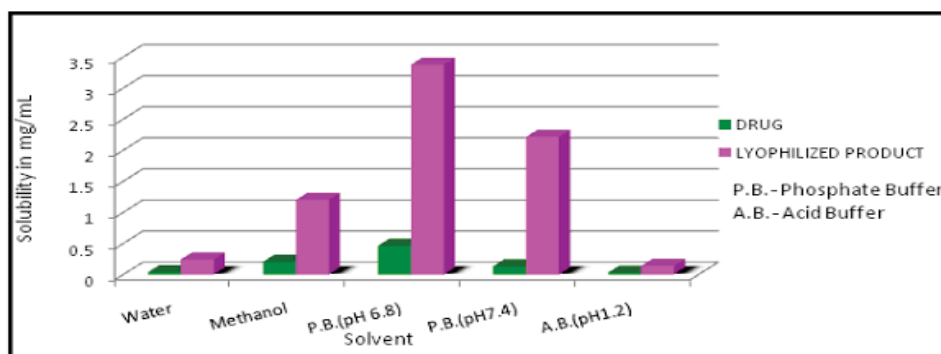
Fig.1: Solubility chart for different solvents.

Storage: Store in cool & dry place, away from sunlight.

III.   HISTORY:

In March 27, 1998 FDA approved sildenafil[6] which was the revolutionary step to treat erectile dysfunction with sales overreach US$ 1 billion. After that, FDA approved verdenafil[9] & tadalafil[8] on August 19, 2003 and November 21, 2003 respectively.

In August 1991 a partnership build between Glaxo and ICOS for the development of new drugs which results the discovery of Tadalafil by Glaxo Smith Kline (that time Glaxo Wellcome). Bothell, Washington biotechnology company ICOS Corporation started studying a compound IC351, which was a phosphodiestrase type 5 enzyme inhibitor, in 1993, but that time they were not testing this compound for treatment of ED, from research they recognized that this compound is useful for the disorder. Then, in 1994, ICOS got patent for the compound IC351 and soon Phase1 Clinical trials started in 1995. Men who were affected from ED initiated for Phase 2 trials, and then further proceed to Phase 3 trials which supported FDA approval of drug.[8,10]

In 1996 Glaxo reverse their agreement with ICOS on partnership of 50-50 profit share, as the drugs developed were not in the company's core markets.

For the further development and purpose of commercialization of Tadalafil in the field of treatment of erectile dysfunction, ICOS and Eli Lily & Company formed the Lily ICOS, LLC, joint venture Company in 1998. After two years, Lily ICOS, LLC, filed new drug application for compound IC351 with the FDA (under brand name Cialis and generic name Tadalafil). After clinical trial testing Lily ICOS reported to American Urological Association in May 2002 that Tadalafil (Cialis) was effective for upto 36 hours, and one year later, FDA approved Tadalafil. When compared to sildenafil, Tadalafil has an advantage over this, that its half-life of 17.5 hour. [10]

Eli Lily and Company bought the ICOS Corporation for 2.3 billion in 2007, which results that Tadalafil (Cialis) owned by Eli Lily and then closed the ICOS operations. It breaks the joint venture between them and approximately 500 employees of ICOS were fired [23].

Once person had surname "Cialis" objected to Eli Lily & Company's so naming the drug, but company successfully managed to convince that the drug's trade name is not related to the surname.

After some years, on 6 October 2011, U.S.FDA approved Tadalafil for the treatment of prostatic hyperplasia (BPH) [11]. It is a condition in males in which the prostate gland becomes enlarged, obstructing the free flow of urine. FDA also approved Tadalafil for the treatment in condition when both ED & BPH co-exist.

IV. PHARMACOKINETIC PROPERTIES:

Bioavailability: It is varies.
Half life: 17.5 hours
Protein binding: 94%
Metabolism: Liver (predominantly)
Excretion: Urine (approx 36%), Feces (approx 61%)[2,5,13]

V. MECHANISM OF ACTION:

Tadalafil is used to treat erectile dysfunction in males. It works by increasing the blood flow in the specific area, which relaxes the smooth muscles present in the wall of blood vessels. It inhibits the cGMP specific phophodiestrase type 5 inhibitor which is responsible for degradation of cGMP in the corpus cavernosum located in the area of penis. Erection in penis during sexual activity is caused by increased blood flow in penis which results from the relaxation of penile arteries and corpus cavernosal smooth muscle. Response is mediated by release of nitric oxide (NO) from endothelial cells and nerve terminals, which stimulates the synthesis of cGMP in smooth muscle cells. cGMP causes smooth muscle relaxation and increased blood flow in the penis area. It enhances erectile function by cGMP production [13,15].

VI. FORMULATION AVAILABLE:

It is a tablet dosage form available in 2.5, 5, 10 & 20 mg[2,13].

VII. SIDE EFFECTS:

Tadalafil has major and minor side effects which are headache, Nausea, heartburn, persistent cough, diarrhea, flushing, dizziness, difficulty in breathing, allergic skin reaction, vision confusion, difficulty in swallowing, uterine bleeding, upper respiratory tract infection, pain in back, sometimes it can cause painful or prolonged erection[4,13,17,18]

These side effects observed in rare situation, but in case symptoms are experienced continuously it is suggested to consult the doctor.

VIII. VARIOUS ANALYTICAL METHODS:

Different analytical methods have been developed for the determination of Tadalafil like UV, HPLC, RP-HPLC, Mass spectroscopy; chromatographic methods etc. and they are validated according to ICH guidelines. All those methods are briefly explained in this article.

❖ **By UV spectroscopy:**

1. *Zamir G.Khan* et al. [24] established four UV-spectrophotometric methods for the determination of tadalafil in bulk & in pharmaceutical formulation. "Method A" was first order derivative UV spectrophotometry using amplitude, "Method B" was first order derivative UV using area under curve technique, "Method C" was second order derivative using amplitude and "Method D" was second order derivative using area under curve technique. These methods had shown best results in term of linearity, accuracy, precision, LOD and LOQ for bulk drug & marketed formulation as well. Tadalafil showed maximum absorbance 284nm in N,N-dimethylformamide. For "Method A" amplitude was recorded at 297nm, for "Method B" AUC was integrated in the range of 290.60-304.40nm. For "Method C" amplitude was 284nm while for "Method D" AUC was in range of 280.80-286.20nm. Tadalafil obeyed Beer-Lambert's law for method A & B in range of 5-50µg/ml, while for method C & D the range was 20-70µg/ml. For all the four methods the correlation coefficients were found to be >than 0.999.

2. *Rahman Ahmed* et al. [25] developed a high sensitive spectrophotometric method for the determination of Tadalafil in pharmaceutical preparations and industrial wastewater samples. Maximum absorbance of Tadalafil was shown at 204nm in 1:1 ethanol-water. Beer's law was obeyed in the range of 1-7µg/ml, with molar absorptivity and Sandell's sensitivity of 0.783×10^5 l/mol.cm and 4.97 ng/cm^2 respectively. Accuracy of the method was 100±0.13 and relative standard deviation was less than 1.7%. The LOD and LOQ were found to be 0.18 & 0.54µg/ml respectively. The proposed method was validated by sensitivity and precision which proves suitability for the routine analysis of tadalafil in true samples.

3. *Gunjan Amin* et al. [26] proposed dual wavelength spectrophotometric method for simultaneous determination of tadalafil in combination dosage form with depoxitine HCl. Method was based on determination at the absorbance difference between 280 & 295.4nm and 255 & 298.2nm for Depoxitine HCl and Tadalafil respectively. For Tadalafil linearity was obtained in the concentration range of 4-24µg/ml and for Depoxitine HCl range was 10-60µg/ml in solution of methanol. The method was successfully applied to pharmaceutical dosage form because no interference from the tablet excipients was found.

4. *Mohammad Yunoos* et al. [27] established a simple, sensitive, precise & highly accurate UV spectrophotometric method developed for the determination of Tadalafil in bulk & tablet dosage form. Maximum absorbance of tadalafil was shown 284nm in

methanol solution. Beer-Lambert's law was obeyed in the concentration range 2-20µg/ml with 1.65×10^4 mol^{-1}cm^{-1}, the slope, intercept, correlation coefficient, detection and quantitation limits were also calculated. This study shows that the method was not affected by the presence of common excipients by the result of percentage recovery and placebo interference.

❖ **By spectrophotometric method:**

1. *Ali Al Kaf* et al. [28] developed two simple spectrophotometric methods for the determination of Tadalafil in pure & tablet dosage form. The methods were based on the formation of ion-pair complexes between the basic nitrogen of the drug with bromocresol purple (BCP) and methyl orange (MO) in acidic buffer solution. Complexes were extracted with chloroform and reported at 410 & 425nm using BCP & MO respectively. Beer's law obeyed in the concentration range 2-20µg/ml with correlation coefficient ≥0.9996. The molar absorptivity, Sandell's sensitivity, LOD & LOQ were also calculated. The composition of the ion-pairs was found 1:1 by Job's method. The proposed methods have been applied successfully for the analysis of TDF in pure and tablet dosage form.

2. *Safwan Farihat* et al. [29] developed simple and reproducible spectrophotometric methods for the determination of Tadalafil (TDF) in pure & pharmaceutical form. Two methods were developed, Method A was based on oxidation of TDF with a known excess amount of Ce(IV) then estimate the unreacted amount of Methyl orange (MO) dye at 507nm. Method B was based on the oxidation of TDF with excess N-bromosuccinamide then estimates the amount of Indigo carmine at 610nm. The methods were linear in the concentration ranges 18-60 and 10-55µg/ml with correlation coefficients of 0.993 & 0.992 and LOD of 10.5 & 5.3µg/ml for the two methods respectively. The proposed were applied for the determination of the drug in pharmaceutical formulations with recovery & relative standard deviations of 97%± 1.4 and 98% ± 1.1 for the two methods. The developed methods are equally accurate, precise and reproducible compared to the official methods.

Table 1: Spectrophotometric methods for the analysis of Tadalafil.

Compounds	Method	Λmax(nm)	Solvent/procedure	LOD (µg/ml)
TDF	First order derivative using amplitude	297	N,N-Dimethylformamide	0.11
	First order using area under curve	290.60-304.40		0.15
	Second order derivative using amplitude	284		0.50
		280.80-286.20		0.49

	Second order derivative using AUC			
TDF, Depoxitine HCl	Dual wavelength spectroscopy	280-295.4(DPH) 255-298.29(TDF)	methanol	0.023(TDF) 0.020(DPH)
TDF	Calibration curve method	284	methanol	-
TDF	Calibration curve method	204	Ethanol:water	0.18
TDF	Formation of two ion-pair complexes	410 425	chloroform	0.092 0.11
TDF	Spectrophotometric method based on oxidation with: Ce(IV) & N-bromosuccinamide	507 610	-	10.5 5.3

❖ **By High Performance Liquid Chromatography (HPLC):**

1. *Sunil R. Dhaneshwar* et al. [30] developed HPLC method for simultaneous determination of Tadalafil & Depoxitine HCl in formulation. This method was based on HPLC separation of the two drugs on the Thermo Hypersil BDS-C_{18}(250mm×4.6mm, 5.0µ) from Germany with isocratic conditions and simple mobile phase containing acetonitrile: 0.1% triethyl amine in water pH adjusted to 4.0 with ortho phosphoric acid (80:20) at flow rate of 1ml/min using UV detection at 229nm. The linear regression analysis data for the calibration plots showed a good linear relationship over the concentration range of 0.25-4µg/ml for Tadalafil and 0.75-12µg/ml for Depoxitine HCl respectively. The mean values of the correlation coefficient, slope and intercept were 0.9995±1.27, 133612±0.72 and 49109± 1.21 for Tadalafil and 0.9992± 0.78, 155825± 0.87 and 132842±0.54 for Depoxitine HCl, respectively. The LOD and LOQ were 0.010µg/ml and 0.025µg/ml for Tadalafil and 0.005µg/ml and 0.020µg/ml for Depoxitine HCl, respectively. Statistical analysis showed that the method is repeatable and selective for the estimation of Tadalafil and Depoxitine HCl.

2. *Divya Yada* et al. [31] developed a simple, reliable and reproducible HPLC method for the analysis of Tadalafil. The column used was YMC-Pack ODS AQ (150mm×4.6mm, i.d.). The mobile phase was phosphate buffer (10mm, pH3.0) acetonitrile gradient run at the flow rate of 1ml/min with UV (PDA) detector at 220nm at ambient temperature. Extraction of Tadalafil was carried out by using methanol. Linearity was observed in the range of 50-150µg/ml with correlation

coefficient 0.99 and 10ng/ml as the limit of detection (LOD). The parameters of validation proved the precision and stability of the method & its applicability for the assay of tadalafil.

3. *Lydia Rabbaa-K*habb az et al. [32] developed a simple & sensitive High performance liquid chromatography (HPLC) method to determine Tadalafil, a selective and reversible phosphodiesterase inhibitor, in human serum. Methyl paraben used as the internal standard. The analyte and internal standard were extracted by a single step liquid-liquid extraction with dichoromethane in alkaline serum. The chromatographic separation was performed on reverse phase LiChrospher 100, C_{18} column (Agilent Technologies) with a mobile phase consisting of acetonitrile-water (7:13) containing 0.1mM glacial acetic acid (pH 2.5-2.7).

Ultraviolet detection was performed at 280nm, detection limit was 1.5ng/ml and LOQ was less than 10ng/ml for Tadalafil. The calibration curves were linear over the concentration range 10-800ng/ml. Accuracy, precision and stability studies were satisfactory. This analytical procedure is relatively inexpensive and simple and is particularly suitable when tandem mass spectrometric detection is not available. This method can be used to determine serum tadalafil concentrations in drug monitoring or in pharmacokinetic investigations.

❖ **By RP-HPLC:**

1. *Jyoti Salgar* et al. [33] aimed another study to develop a simple method for estimaton of tadalaafil in tablet dosage form. Analyte was separated on Phenomenex Luna C_{18} column (150×4.6mm, 5μ) with security guard cartilage C_{18} using acetonitrile: water (50:50) as a mobile phase at flow rate 1.0ml/min and detection was carried out at 286nm. The linearity was found to be in the range of 0.25-5μg/ml with regression coefficient 0.999, elution time was 4.6min. The method was validated for precision, robustness and recovery.

2. *Bojanapu A.* et al. [34] developed a novel, precise and sensitive RP-HPLC method for the validated estimation of Tadalafil in bulk and tablet dosage form. The separation was achieved on Agilent Eclipse XDB C_{18} column (150mm×4.6mm, 5μ) using a mobile phase that consists of the potassium dihydrogen orthophosphate and acetonitrile in the ratio of 50:50, pH 6 was adjusted with orthophosphoric acid. The flow rate was maintained at 1.2ml/min and the detection wavelength was 285nm, retention time was found to be 3.181min. The linearity was in the range of 10-150μg/ml & correlation coefficient was 0.999. The %RSD was satisfactory which showed the method found to be reliable.

3. *A. Chenthilnathan* et al. [35]developed a simple, efficient and reproducible RP-HPLC method for simultaneous determination of Tadalafil and Depoxitine HCl in combined pharmaceutical solid dosage form. The separation was carried out on Hypersil BDS

C8, 250× 4.6mm column using buffer (6.8g of potassium dihydrogen orthophosphate and 0.94g of sodium hydroxide in 900ml water, adjusted to pH 6.8 with ortho phosphoric acid): acetonitrile in the ratio 55:45 v/v as eluent. The flow rate was 1.0ml/min and effluent was detected at 254nm. The retention time of tadalafil was 4.473min & percentage recovery was within the range between 99.88% - 100.37%. The linear range was found to be 50-150μg/ml & regression coefficient was 0.9984 for tadalafil. The percentage relative standard deviation for accuracy & precision was found to be less than 2%. Hence, the method could be successfully applied for routine analysis.

4. *Sadhana J. Rajput* et al.[36] developed an isocratic HPLC method and two classical UV spectrophotometric methods i.e. first derivative zero crossing point method (FDZCP) and ratio derivative spectrophotometry (RDS) for the simultaneous estimation of Ambrisentan (AMB) and Tadalafil (TDF) in its laboratory sample. The HPLC method was developed using Phenomenex Luna C18 column (250×4.6mm, 5μ particle size), with mobile phase composition of methanol: acetonitrile: ammonium acetate buffer pH 6 (35:30:35). The flow rate was 1ml/min and effluent was detected at 264nm. The retention time for TDF was 5.97min. All the three methods were successfully applied for the analysis of laboratory mixture. The recovery for TDF was found to be 98.04-101.63% by HPLC method, 99.73-102.98% by FDZCP method and 100.36-102.11% by RDS method. One way ANOVA was performed and it was found that there is no significance difference between the results of the three methods i.e. the UV spectrophotometric methods were found to be as good as HPLC method and hence any of these methods can be used for the simultaneous estimation of AMB and TDF.

5. *Ahmed R.Al* et al. [37] developed a simple, specific, precise and accurate RP-HPLC method for simultaneous estimation of esomeprazole and tadalafil in pharmaceutical formulation. The separation was achieved by Hypersil BDS C_{18} column (250mm×4.6mm; 5μm) using acetonitrile: 0.05M potassium dihydrogen phosphate buffer at pH 6 adjusted with phosphoric acid at a flow rate of1ml/min. Detection was carried out at wavelength 285nm, retention time for esomeprazole & tadalafil were 3.1, 3.7min, respectively. The mean recoveries were found to be in the ranges of 98-102%. The proposed method has been validated as per ICH guidelines and successfully applied to the simultaneous estimation of esomeprazole & tadalafil in pharmaceutical formulation.

6. *B.Prasanna Reddy* et al. [38] described the development of a stability indicating RP-HPLC method for the analysis of Tadalafil. The sample separated on an Inertsil $C_{18,}$ (5μ, 150mm×4.6mm i.d) by isocratic run using acetonitrile and phosphate buffer as mobile phase, with a flow rate of 0.8ml/min, and the determination wavelength was 260nm for the analysis. The described method was linear within range of 70-130μg/ml and shows correlation coefficient of 0.999. Tadalafil was exposed to acidic, basic,

oxidative and thermal stress conditions & the stressed samples were analyzed by the proposed method. Chromatographic peak purity results indicated the absence of coeluting peaks with the main peak of tadalafil, which demonstrated the specificity of assay method for estimation of tadalafil in presence of degradation products.

7. *Alivelu Samala* et al. [39] proposed a simple and accurate RP-HPLC method for the estimation of Tadalafil in tablet dosage form. A sharp peak obtained at 3.068 min, on Agilent Eclipse XBD C_{18} column (150×4.6mm i.d., 5µm particle size) with mobile phase, acetonitrile and buffer solution (50:50v/v), delivered in isocratic mode at a flow rate of 1.2ml/min at 282nm, methanol was used as diluents. Linearity was found to be in the range of 10-150µg/ml and shows a correlation coefficient of 0.999. Accuracy was determined by recovery studies from tablet dosage form & ranges from 100.3-100.8%. The intraday and interday precision was found to be 0.54% and 0.52% respectively.

❖ By LC-MS:

1. *Pranav S. Shrivastav* et al. [40] developed a highly sensitive LC-MS/MS method for the determination of Tadalafil in human plasma. TDF and its deuterated internal standard, tadalafil-d3, were extracted from 200µL plasma using Phenomenex Strata-X-C 33µ extraction cartridges. Chromatographic analysis was carried out on Synergi Hydro-RP C_{18} column with a mobile phase consisting of methanol and 10mM ammonium formate, pH 4 (90:10, v/v), delivered at flow rate 0.9ml/min. Quantitation of the protonated analyte was done on a triple quadrupole mass spectrometer using multiple reaction monitoring via electro spray ionization. The precursor to product ions transitions monitored for TAD and TAD-d3 were m/z 390.3 - 268.2 and m/z 393.1 - 271.2, respectively. The calibration curve was linear over the concentration range of 0.50– 500ng/mL with correlation coefficient, r2 Z 0.9994. Acceptable intra-batch and inter-batch precision(r 3.7%) and accuracy (97.8%to104.1%) were obtained at five concentration levels. The recovery of TAD from spiked plasma was highly precise and quantitative (98.95%to100.61%). Further, the effect of endogenous matrix components was minimal. TAD was found to be stable under different storage conditions in human plasma and also in whole blood samples. The validated method was successfully used to determine TAD plasma concentration in a bioequivalence study with 20mg TAD tablets in 24 healthy volunteers. Method performance was evaluated by reanalyzing 115 study samples.

2. *Sang-Heon Cho* et al. [41] developed & validated a rapid and reliable UPLC-MS/MS method for the determination of tadalafil in human plasma. The samples were deproteinized with acetonitrile. Chromatographic separation was performed on a Shiseido C18 (100×2.1mm, 2.7µm) column with isocratic elution using 2.0mM ammonium acetate and acetonitrile (55:45, v/v) with 0.1% formic acid at a flow rate of 0.7ml/min, total rum time was 1min per sample. The quantitative analysis was

performed using multiple reactions monitoring at transition of m/z 390.4 → 268.3 for tadalafil and m/z 475.3 → 283.3 for sildenafil as an internal standard. The method was fully validated over a concentration range of 5–1,000 ng/mL with a lower quantification limit of 5 ng/mL. Intra and inter-day precision (%RSD) were within 8.4% and accuracy (%RE) was lower than -3.2%. The developed and validated method was successfully applied to a pharmacokinetic study of tadalafil (20 mg) in Korean healthy male subjects.

IX.   CLINICAL STUDIES:

Different clinical studies estimated on the drug –Tadalafil, to check the efficacy and safety of the drug. The studies are briefly given in this article below:

1. *Ahmet Sevki Taskiran* et al. [42] investigate the effects of Tadalafil on nociception, morphine analgesia and tolerance. In this study, 54 Wistar Albino (230-250 g) male rats were used. First of all, four different doses (2, 4, 8, 16 mg/kg) were used to determine the optimum effective dose of tadalafil on nociception. Optimum activity was found at 8 mg/kg and animals were divided into six groups: Saline(S), 8mg/kg tadalafil, 5mg/kg morphine (M), M+ tadalafil, morphine tolerance (MT) and MT+ tadalafil. Saline was given to the control group, tadalafil intraperitoneally and morphine subcutaneously administered at the indicated doses. To develop tolerance to morphine, 10mg/kg morphine was injected daily in the morning and evening for five days and tolerance was evaluated with single dose of morphine on sixth days. The resulting analgesic effect was measured with hot plate and tail flick analgesia tests and recorded at 30th, 60th, 90th and 120th minutes. Tadalafil showed anti-nociceptive effect when given alone at different doses ($p<0.05$). However, tadalafil significantly decreased the analgesic effect of morphine ($p<0.05$). In addition, tadalafil significantly increased the tolerance to morphine ($p<0.05$).
The PDE5 inhibitor tadalafil have anti-nociceptive properties and it decreases analgesic effect of morphine, in addition improves tolerance development. These effects probably may occur via NO/cGMP pathway.

2. *Zhufeng Peng* et al. [43] compared the efficacy & safety between Tadalafil once-a-day and on-demand dosing regimen in patients with ED. A systematic search of Medline, Embase, and Cochrane Library was performed to identify all randomized controlled trials (RCTs) that compared tadalafil used a once-a-day with an on-demand dosing regimen for erectile dysfunction. Six RCTs involving a total of 1,534 patients were included in this review. All studies
Reported the International Index of Erectile Function-Erectile Function domain score and the results of the meta analysis showed no difference between the groups. The overall pooled estimated weighted mean differences (WMD) was 0.97 (95% CI –0.37 to 2.32; $p = 0.16$). Meta-analyses of Sexual Encounter Profile questions 2 and 3 (SEP-2 and SEP-3) showed that the once-a-day dosing regimen was superior to the on-demand regimen with statistical significance. The
WMD of SEP-2 and SEP-3 were 10.32 (95% CI 3.16–17.48; $p = 0.005$) and 11.07 (95% CI 2.57–19.56; $p = 0.01$), respectively. Both dosing regimens of tadalafil

showed similar complication rates. The meta-analyses of adverse events showed no significant differences. The efficacy rates of tadalafil once-a-day and on-demand were similar. No significant difference in safety was found between the 2 dose regimens of tadalafil.

3. *Sandro La Vignera* et al. [44] evaluated the effects of pharmacological treatment with Tadalafil 5mg daily on symptoms and quality of sperm parameters in selected patients with amicrobic Male Accessory Gland Inflammation (MAGI). 120 patients with amicrobic MAGI (mean age 27.0 ± 6.0 years) with mild-moderate ED (erectile dysfunction) according to IIEF-5 (International Index of Erectile Function 5 Items) scores underwent pharmacological treatment with Tadalafil 5 mg daily for six months. Before and after treatment these patients were evaluated through IIEF-5, semen analysis (according to WHO Criteria, 2010), SI-MAGI (Structured Interview about Male Accessory Gland Inflammation), and ultrasound evaluation. Patients with PVE (prostate-vesciculo-epididymitis) showed a significant increase in the percentage of spermatozoa with total (16.0 ± 8.0 versus $30.0 \pm 6.0\%$) and progressive motility (8.00 ± 10.0 versus $25.0 \pm 6.00\%$). It was a significant reduction of the number of patients with complicated ultrasound forms (30.0 versus 52.0) and a significant increase of the number of patients with uncomplicated ultrasound form (90.0 versus 68.0). Finally, there was a significant reduction in the percentage of patients with alterations of sexual function different from DE, such as premature ejaculation (4.00 versus 8.00%), painful ejaculation (4.00 versus 10.0%), delayed ejaculation (12.50 versus 8.00%), and decreased libido (10.0 versus 25.0%).

4. *Mehmet Karabakan* et al. [45] investigate the effect of 5mg daily Tadalafil treatment of the ejaculation time, erectile function and lower urinary tract symptoms in patients with ED. A total of 60 patients diagnosed with erectile dysfunction were retrospectively evaluated using the international index of erectile function questionnaire-5 (IIEF-5), intravaginal ejaculatory latency time (IELT) and international prostate symptoms scores (IPSS). After the patients were treated with 5mg tadalafil once a day for three months, their erection, ejaculation and LUTS were assessed again. The fasting levels of blood glucose, total testosterone, low-density lipoprotein cholesterol, high density lipoprotein cholesterol and total cholesterol were measured. The independent samples t-test was used to compare the pre- and post-treatment scores of the patients. The mean age of the 60 participants was 50.4 ± 7.9 and the mean baseline serum total testosterone, total cholesterol, and fasting blood sugar were 444.6 ± 178.6ng dL-1, 188.7 ± 29.6mg/dL-1,104 (80-360) mg dL-1, respectively. The mean baseline scores were 2.2 ± 1.4 min for IELT, 9.5 ± 3.7 for IIEF-5 and 14.1 ± 4.5 for IPSS. Following the three-month daily 5mg tadalafil treatment, the scores were found to be 3.4 ± 1.9 min, 16.1 ± 4.7, and 10.4 ± 3.8 for IELT, IIEF and IPSS, respectively. When the baseline and post-treatment scores were compared, a statistically significant increase was observed in the IELTs and IIEF-5 values whereas there was a significant decrease in IPSS ($p<0.01$). A daily dose of 5mg tadalafil can be safely used in the treatment of erectile dysfunction and LUTS, which prolongs the ejaculatory latency time.

5. *Joseph A.Califano* et al. [46] determined that PDE5 inhibitors can augment immune function in patients with head and neck cancer through inhibition of myeloid-derived suppressor cells (MDSC). They performed a randomized, prospective, double blinded, placebo controlled, phase II clinical trial to determine the in vivo effects of systemic PDE5 inhibition on immune function in patients with head and neck squamous cell carcinoma (HNSCC).

Tadalafil augmented immune response, increasing ex vivo T-cell expansion to a mean 2.4-fold increase compared with 1.1-fold in control patients (P ¼ 0.01), reducing peripheral MDSC numbers to mean 0.81-fold change compared with a 1.26 fold change in control patients (P¼0.001), and increasing general immunity as measured by delayed type hypersensitivity response (P ¼ 0.002). Tumor-specific immunity in response to HNSCC tumor lysate was augmented in tadalafil-treated patients (P ¼ 0.04). These findings demonstrate that tadalafil augments general and tumor-specific immunity in patients with HNSCC and has therapeutic potential in HNSCC. Evasion of immune surveillance and suppression of systemic and tumorspecific immunity is a significant feature of head and neck cancer development. This study demonstrates that a PDE5 inhibitor, tadalafil, can reverse tumor-specific immune suppression in patients with head and neck cancer, with potential for therapeutic application.

6. *Ali Hamidi Madani* et al. [47] evaluated the safety & efficacy of Tadalafil on lower urinary tract symptoms suggestive of benign prostatic hyperplasia (BPH) in patients treated with standard medication. In this case-controlled randomized clinical trial on 132 patients with obstructive and irritative urinary tract symptoms due to BPH, IPSS ≥ 8, no indication for surgical intervention and that reached plateau levels of response to treatment were selected. These patients were randomly allocated in two groups (each containing 66 patients). The treatment group received standard treatment of BPH and tadalafil (10 mg nightly); the placebo group received only standard treatment of BPH. IPSS, maximum urinary flow rate (Qmax) and quality of life were assessed before and after a 3-month period of study. Before treatment, mean IPSS, Qmax and quality of life values in the treatment and placebo groups were 13.06 •} 4.37 and 13.66 •} 4.25, 8.92 •} 2.96 mL/s and 9.09 •} 2.91 mL/s, 2.93 •} 0.86 and 2.66 •} 0.78, respectively. After treatment, mean IPSS, Qmax, and quality of life values in treatment group were 7.66 •} 3.99, 9.99 •} 4.76 mL/s and 1.80 •} 0.98, respectively. These findings were compared to corresponding values of the placebo group (11.37 •} 3.64, 8.73 •} 2.22 mL/s and 2.19• } 0.53, respectively): IPSS and quality of life were significantly different but Qmax didn't show a significant change.

Tadalafil improves quality of life and urinary symptoms in patients with LUTS suggestive of BPH, but doesn't have any significant effect on Qmax. Therefore, this drug may be effectively used in combination with standard medical therapies for BPH.

7. *George Groeneweg* et al. [48] investigated the effect of the PDE5 inhibitor tadalafil on the microcirculation in patients with cold Complex Regional Pain Syndrome (CRPS) in one lower extremity by double-blind, randomized and controlled trial. Twenty-four patients received 20 mg tadalafil or placebo daily for 12 weeks. The patients also participated in a physical therapy program. The primary outcome measure was temperature difference between the CRPS side and the contralateral side, determined by measuring the skin temperature with videothermography. Secondary outcomes were: pain measured on a Visual Analogue Scale, muscle force measured with a MicroFet 2 dynamometer, and level of activity measured with an Activity Monitor (AM) and walking tests. At the end of the study period, the temperature asymmetry was not significantly reduced in the tadalafil group compared with the placebo group, but there was a significant and clinically relevant reduction of pain in the tadalafil group. Muscle force improved in both treatment groups and the AM revealed small, non-significant improvements in time spent standing, walking, and the number of short walking periods. Tadalafil may be a promising new treatment for patients that have chronic cold CRPS due to endothelial dysfunction, and deserves further investigation.

8. *Carlo Baldari* et al. [49] from Units of Endocrinology, University of Rome, investigated that whether a single oral long half-life phosphodiesterase type 5 inhibitor (tadalafil) administration influences the HPA axis response to exercise-related stress. This was a double blind, cross over trial. Nine healthy male athletes include as participants. All subjects performed a maximal exercise test in normoxia, after which they receiveda single oral administration of tadalafil or placebo. Then after a 2-wk washout period, they were crossed over and repeated the exercise test. Each subject was his own control. Salivary collections, for steroid evaluations [cortisol, dehydroepiandrosterone sulphate (DHEAS), testosterone] and respective ratio calculation (DHEAS to cortisol, testosterone to cortisol, testosterone to DHEAS), were performed before each exercise (Pre-Ex), immediately after (Post-Ex), and at 30 min during recovery. As expected, mean salivary cortisol concentration increased immediately after exercise after both tadalafil and placebo (P _ 0.014 and P _0.036 *vs.* Pre-Ex, respectively); however, the cortisol increase was significantly higher after tadalafil administration (P_0.034 *vs.* placebo). Furthermore, an increased salivary testosterone after exercise was observed only after tadalafil administration (P _0.029 *vs.* Pre-Ex). No effects of either exercise and/or tadalafil administration on salivary DHEAS concentrations were observed. DHEAS to cortisol and testosterone to cortisol ratios significantly decreased after exercise after tadalafil administration (P _ 0.037, and P _ 0.02 *vs.* placebo, respectively).The final conclusion from the results was that Tadalafil administration amplified the salivary cortisol and testosterone responses to a maximal exercise-related stress in healthy trained humans.

9. *Inigo Saenz De Tejada* et al. [50] evaluate the efficacy and safety of Tadalafil taken as needed before sexual activity by men with diabetes and erectile dysfunction. Men with type 1 or type 2 diabetes and a minimum 3-month history of ED were randomly allocated to one of three groups: placebo (*n* _71), tadalafil 10 mg (*n* _ 73), or tadalafil 20 mg (*n* _ 72) taken up to once daily for 12 weeks. Changes from baseline in mean scores on the erectile function domain of the International Index of Erectile Function (IIEF) and changes from baseline in the proportion of "yes" responses to question 2, "Were you able to penetrate?," and 3, "Were you able to complete intercourse?," of the Sexual Encounter Profile were coprimary outcome measures.

A total of 191 (88%) of 216 patients completed the study. Treatment with tadalafil significantly improved all primary efficacy variables, regardless of baseline HbA1c level. Therapy with tadalafil also significantly improved a number of secondary outcome measures, including changes in other IIEF domains, individual IIEF questions, and percentage of positive responses to a global assessment question measuring erection improvement. Treatment with tadalafil did not alter mean HbA1c levels. Tadalafil was well tolerated, with headache and dyspepsia being the most frequent adverse events with active treatment.

Conclusion was that Tadalafil therapy significantly enhanced erectile function and was welltolerated by men with diabetes and ED.

X. **DISCUSSION:**

It is discussed that Tadalafil belongs in the category of Erectile Dysfunction (ED) drug which is soluble in organic solvents. Selection of solvents would not be problematic in analysis of Tadalafil as there are concerns with various composition of organic phase. The review covers the current analytical methods for estimation of Tadalafil and its combination pharmaceutical sample, estimation of individual drugs in the multi dosage forms preferred RP-HPLC methods and also covers the clinical studies regarding its efficacy & safety on different patients with different aspects.

XI. **CONCLUSION:**

A wide range of techniques are available for analysis of Tadalafil in pharmaceutical samples. Reported data revealed that RP-HPLC was extensively used for determination of the drug. For analysis of Tadalafil in pharmaceuticals, HPLC with UV is applicable because this method provides accurate, precise & low cost compared to other advanced techniques. Clinical data shows the studies carried out on efficacy and safety of the drug with various aspects. Furthermore investigations are needed regarding analysis & clinical studies of the drug for the benefit of humans.

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