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A REVIEW ON ANALYTICAL METHOD DEVELOPMENT FOR SIMULTANEOUS ESTIMATION AND VALIDATION OF FINASTERIDE AND TADALAFIL

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ABSTRACT

Finasteride is a 5α-reductase inhibitor and therefore an antiandrogen. It works by decreasing the production of dihydrotestosterone (DHT) by about 70%, including in the prostate gland and the scalp. In addition to DHT, finasteride also inhibits the production of several anticonvulsant neurosteroids including allopregnanolone, and rostanediol, and THDOC (Tetrahydroxycorticosteroid). Finasteride has been used for the treatment of symptomatic benign prostatic hyperplasia (BPH) in men with an enlarged prostate and for the treatment of male pattern hair loss (androgenetic alopecia) in men. Finasteride may improve the symptoms associated with BPH such as difficulty urinating, getting up during the night to urinate, hesitation at the start and end of urination, and decreased urinary flow. Tadalafil is a PDE5 inhibitor which increases blood flow to the penis. It also dilates blood vessels in the lungs, which lowers the pulmonary artery pressure. Used to treat erectile dysfunction (ED), benign prostatic hyperplasia (BPH), and pulmonary arterial hypertension. Combination of tadalafil and finasteride is a safe, effective, and well tolerated treatment for BPH**.** This combination may be particularly effective in reducing treatment-related sexual adverse events associated with 5α-reductase inhibitor. This review includes various analytical methods for simultaneous estimation of finasteride and tadalafil. Various analytical methods were reported for determination of finasteride and for Tadalafil in bulk drug or in combination with other drugs. Till date there is only one UV spectrophotometric method and

only one LC/MS method was reported for Finasteride and tadalafil in combined dosage form. This review can be used for further analytical method development.

KEYWORDS: Benign Prostatic Hyperplasia (BPH), Finasteride, Tadalafil, Analytical Methods, Development, Validation.

INTRODUCTION

Benign prostatic hyperplasia (BPH) is characterized by progressive enlargement of the prostate gland, which results in obstruction of urine outflow from the bladder. It is a common condition as men get older. An enlarged prostate gland can cause also cause bladder, urinary tract or kidney problems. The prostate gland is located beneath your bladder. The tube that transports urine from the bladder out of your penis (urethra) passes through the center of the prostate. When the prostate enlarges, it begins to block urine flow. Most men have continued prostate growth throughout life. In many men, this continued growth enlarges the prostate enough to cause urinary symptoms or to significantly block urine flow.[1]

The combination of finasteride and tadalafil was approved by FDA in December 2021 with brand name ENTADFI to treat urinary tract symptoms caused by an enlarged prostate, also called BPH with low potential for adverse sexual side effects.

Finasteride is a steroidal molecule. Finasteride is a Type 2, 5 alpha reductase inhibitor. Type 2, 5 alpha reductase is an intracellular enzyme that converts the androgen testosterone into 5a-dihydrotestosterone hormone responsible for prostate growth. It has anti-androgenic properties. Finasteride prevents the peripheral conversion of testosterone to dihydrotestosterone (DHT), which lowers serum DHT levels, increases hair development, and slows hair loss by bringing down the concentration of DHT in the scalp to levels found in hairy scalps.[2]

Tadalafil is a selective phosphodiesterase 5 (PDE5) inhibitor which is used to treat mild to severe erectile dysfunction in man. It is an impotence agent. Phosphodiesterase 5 (PDE5) inhibitor is responsible for the degradation of cGMP in the corpus cavernosum located around the penis. Penile erection during sexual stimulation is caused by increased penile blood flow resulting from the relaxation of penile arteries and corpus cavernosal smooth muscle. This response is mediated by the release of nitric oxide (NO) from nerve terminals and endothelial cells, which stimulates the synthesis of cGMP in smooth muscle cells. Cyclic

GMP causes smooth muscle relaxation and increased blood flow into the corpus cavernosum. The inhibition of phosphodiesterase type 5 (PDE5) by tadalafil enhances erectile function by increasing the amount of Cgmp.[3]

Physical and Chemical properties

1. Finasteride

Finasteride is white solid crystalline powder. Its chemical name is 17 beta-(N-tert-butylcarbamoyl)-4-aza-5α-androst-1-en-3-one. Its molecular weight is 372.6 gm/mol. Its molecular formula is $C_{23}H_{36}N_2O_2$. Freely soluble in ethanol and Methylene Chloride and in dichloromethane; Practically insoluble in water. Its melting point is near 257 °C. Its log P (partition co-efficient) is 3.03. It is official in Indian Pharmacopoeia, United States Pharmacopoeia and Japan Pharmacopoeia.[4,5,6]

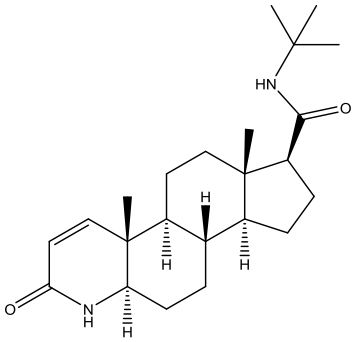

Figure 1: Chemical structure of finasteride.

2. Tadalafil

Tadalafil is solid white or almost white powder. Its chemical name is (6R,12aR)-6-(1,3-benzodioxol-55-yl)-2-methyl-2,3,6,7,12,12a-hexahydropyrazinol[1',2':1,6]-pyrido[3,4-b] indole-1,4-dion. Its molecular weight is 389.4 gm/mol. Its molecular formula is $C_{22}H_{19}N_3O_4$. Freely soluble in dimethyl sulfoxide; slightly soluble in dichloromethane; Practically insoluble in water. Its melting point is near 302-303 °C. Its log P (partition co-efficient) is 2.89. It is official in Indian Pharmacopoeia and United States Pharmacopoeia.[4,5,7]

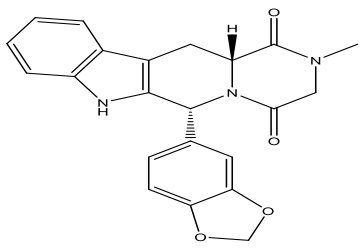

Figure 2: Chemical structure of tadalafil.

Introduction to analytical method Development and Validation

Analytical chemistry is the study of separation, quantification and chemical components identification of natural and artificial materials constituted with one or more compounds or elements. Analytical chemistry is separated into two main categories, qualitative analysis that is to say the identification with regard to the chemical components exist in the sample, whereas quantitative analysis estimates the amount of certain element or compound in the substance i.e., sample.

Drug analysis is the basis for the determination of the product. Due to potential concerns in the ongoing and widespread use of these medications, reports of additional side effects/ new toxicity, the development of patient resistance, and the introduction of better medications by competitors, very often, there is a time lag between the date of introduction of a drug in to the market to the date of its inclusion in pharmacopeia's. Under these conditions, standard and analytical procedures for these drugs may not be available in pharmacopeias. Therefore, it becomes necessary to develop new analytical methods for such drugs.

The analytical method development and validation is essential for analytical method development and tested extensively. Analytical method validation is establishing documented evidence which provides a high degree of assurance that specific processes consistently produce a product meeting its predetermined specifications and quality attributes. The objective of validation of an analytical procedure is to demonstrate that it is suitable for its intended purpose. The validation parameters which are tested include specificity, linearity, accuracy, precision, range, detection limit, quantization limit, and robustness.[8,9]

Various analytical methods like Spectrophotometric, RP- HPLC and HPTLC were reported for estimation of finasteride in bulk and in combination with other drugs like Minoxidil, Tamsulosin, Depoxetin etc. Similarly, for estimation of Tadalafil, various methods like spectrophotometric, HPLC, and HPTLC methods were reported in bulk and in combination with other drugs like Tamsulosin, Depoxetin, Mecitentan, Silodosin etc. Till date, only one spectrophotometric method and one LC-MS/MS method were reported for simultaneous estimation of Finasteride and Tadalafil in combined dosage form. There is no HPLC or HPTLC method was reported for simultaneous estimation of Finasteride and Tadalafil combined dosage form.

Literature review of finasteride

- **Pharmacopeial methods for estimation of finasteride**

Sr. No.	Drug/ Formulation	Method	Official in	Description	Ref. No.
1.	Bulk drug	HPLC	IP 2018	**Stationary phase:** A stainless steel column 25 cm x 4.6 mm packed with base deactivated end capped octadecylsilane bonded to porous silica or ceramic micro particles (5 µm). **Mobile phase:** A mixture of 10 volumes of acetonitrile, 10 volumes of tetrahydrofuran and 80 volumes of water. **Flow rate:** 1.5 ml/min **Wavelength:** 210 nm	[4]
2.	Tablet	HPLC	IP 2018	**Stationary phase:** A stainless steel column 5 cm x 4.6 mm packed with octadecylsilane bonded to porous silica (3 µm). **Mobile phase:** A mixture of 42 volumes of water and 58 volumes of acetonitrile. **Flow rate:** 1.5 ml/min **Wavelength:** 220 nm	[4]
3.	Tablet	HPLC	IP 2018	**Stationary phase:** A stainless steel column 25 cm x 4.0 mm packed octadecylsilane bonded to porous silica (5 µm). **Mobile phase:** A mixture of 10 volumes of acetonitrile, 10 volumes of tetrahydrofuran and 80 volumes of water. **Flow rate:** 1.5 ml/min **Wavelength:** 210 nm	[4]
4.	Tablet	HPLC	IP 2018	**Stationary phase:** A stainless steel column 10 cm x 4.6 mm packed octadecylsilane bonded to porous silica (5 µm). **Mobile phase:** A mixture of equal volumes of acetonitrile and 0.0025 M of orthophosphoric acid. **Flow rate:** 1.5 ml/min **Wavelength:** 240 nm	[4]
5.	Bulk drug	LC	USP 2019	**Stationary phase:** 4.6 mm × 30 cm; 4 µm packing L1 **Mobile Phase:** Filtered and degassed mixture of water: tetrahydrofuran: acetonitrile (8:1:1	[10]

				v/v/v) **Flow rate:** 1.5 ml/min **Wavelength:** 210 nm **Column temperature:** 60 °C **Injection volume:** about 15 µl	
6.	Bulk drug	LC	USP 2019	**Stationary phase:** 3.0 mm × 3.0 cm; 3 µm packing L7 **Mobile Phase:** Filtered and degassed mixture of water: tetrahydrofuran (4:1 v/v) **Flow rate:** 3 ml/min **Wavelength:** 215 nm **Injection volume:** about 10 µl	[10]
7.	Tablet	LC	USP 2019	**Stationary phase:** 4.6 mm × 15 cm; packing L11 **Mobile Phase:** Filtered and degassed mixture of acetonitrile: water (11:9 v/v) **Flow rate:**1.5 ml/min **Wavelength:** 220 nm **Column temperature:** 45 °C **Injection volume:** about 100 µl	[10]
8.	Tablet	LC	USP 2019	**Stationary phase:** 4.6 mm × 10 cm; 4 µm packing L1 **Mobile Phase:** Filtered and degassed mixture of 2.5 mm phosphoric acid: acetonitrile (1:1 v/v) **Flow rate:**1.5 ml/min **Detection:** 240 nm **Column temperature:** 45 °C **Injection volume:** about 20 µl	[10]
9.	Bulk drug	LC	BP 2010	**Stationary phase:** 0.25 m × 4 mm end capped octadecylsilyl silica gel **Mobile Phase:** Acetonitrile: Tetrahydro furan: Water (10:10:80 v/v/v) **Flow rate:**1.5 ml/min **Column temperature:** 60 °C **Injection volume:** 15 µl	[11]

- **Reported method for estimation of finasteride**

Sr. No.	Method	Description	Ref. No.
1.	Validated HPTLC method for the simultaneous determination of alfuzosin, terazosin, prazosin, doxazosin and finasteride in	**Stationary Phase:** Precoated TLC silica gel aluminium plates $60F_{254}$ **Mobile Phase:** Methylene chloride: n-hexane: Methanol (8.8:0.3:0.9 v/v/v) **R_f:** 0.69	[12]

	pharmaceutical formulations	**Wavelength:** 254 nm	
2.	Stability Indicating Method Development and Validation of Finasteride by High-Performance Thin-Layer Chromatography Studies	**Stationary Phase:** TLC plates pre-coated with silica gel 60 F_{254} (10 cm × 10 cm with 250 mm layer thickness) **Mobile Phase:** chloroform-methanol (8:2 % v/v) **R_f:** 0.57 **Wavelength:** 210 nm	[13]
3.	Determination of Finasteride in Tablets by High Performance Liquid Chromatography	**Stationary Phase:** ODS C18 reversed phase column **Mobile Phase:** Methanol: Water (80:20 v/v) **Retention time:** 6.1 min **Wavelength:** 225nm **Flow rate:** 1 mL/min	[14]
4.	Development and validation of a RP- HPLC method for determination of finasteride in pharmaceutical dosage forms	**Stationary Phase:** C18 column, Inertsil ODS 3v (C8, 4.6×250mm, 5µm.) **Mobile Phase:** Acetonitrile: Water (60:40 v/v) **Retention time:** 3.71min **Wavelength:** 254 nm **Flow rate:** 1.10 ml/min	[15]
5.	LC determination of finasteride and its application to storage stability studies	**Stationary Phase:** Shimpak C8 column (5µm, 15.0 cm x 4.6 mm) **Mobile Phase:** Acetonitrile: Water (95:05 v/v) **Retention time:** Less than 10 min **Wavelength:** 210 nm **Flow rate:** 0.7 ml/min	[16]
6.	Rapid Analysis of Finasteride in Bulk and Formulations by Rp-HPLC-PDA Method	**Stationary Phase:** Phenomenex C18 column (150 mm x 4.6 mm, 5.0 µ particle size) **Mobile Phase:** 0.02% formic acid (in water): methanol in the ratio of 20:80 (v/v) **Retention time:** 3.4 min **Wavelength:** 220 nm **Flow rate:** 1mL/min	[17]
7.	A Stability-Indicating HPLC Method to Determine Finasteride in A Tablet Formulation	**Stationary Phase:** C18 column **Mobile Phase:** Methanol: Water (70: 30 v/v) **Wavelength:** 210 nm **Flow rate:** 1 mL/min	[18]

8.	A Stability Indicating UPLC Method for Finasteride and Its Related Impurities	**Stationary Phase:** Waters ACQUITYTM UPLC BEH Phenyl Column (150 mm × 2.1 mm, 1.7 µm) **Mobile Phase:** Solution A and B as mobile phase. The solution A Contains 2.5 mM ortho phosphoric acid (Buffer) and solution B contains a mixture of acetonitrile and water in the ratio of (90:10 v/v) **Wavelength:** 210nm **Flow rate:** 0.22 ml/min	[19]
9.	Development and Validation of UV Spectrophotometric Method for the Estimation of Finasteride in Tablets	**Solvent:** Dichloromethane **Wavelength:** 254 nm **Linearity:**5 – 25 µg/mL **R^2:**0.9986 **LOD:** 1.138µg/ml **LOQ:** 3.448 µg/ml **% Recovery:** 98.82 – 102.11%	[20]

- **Reported methods for estimation of finasteride with other drugs**

Sr. No.	Method	Description	Ref. No.
1.	HPTLC Method Validation for simultaneous determination of Tamsulosin Hydrochloride and Finasteride in Bulk and Pharmaceutical Dosage Form	**Stationary Phase:** Precoated silica gel aluminium plate 60 F (20 cm x 10 cm with 0.2 mm thickness) **Mobile Phase:** Toluene: n-propanol: Triethylamine (3.0:1.5:0.2 v/v) **R_f:** **Finasteride:** 0.60 **Tamsulosin Hydrochloride:** 0.35 **Wavelength:** 190 - 400 nm	[21]
2.	Stability Indicating Planar Chromatographic method for Estimation of Minoxidil and Finasteride Combination used in the treatment of Hair loss	**Stationary Phase:** HPTLC plates precoated with silica gel 60 F_{254} **Mobile Phase:** n-butanol: TEA (10:0.1 v/v) **R_f:** **Finasteride:** 0.63 ± 0.65 **Minoxidil:** 0.22 ± 0.24 **Wavelength:** 223 nm	[22]
3.	Simultaneous Estimation of Finasteride and Tamsulosin Hydrochloride in Pharmaceutical Dosage Forms by UV Spectrophotometry, RP HPLC and HPTLC	**Method: HPTLC** **Stationary Phase:** HPTLC Silica gel 60F_{254} precoated (Merck) **Mobile Phase:** Toluene: Chloroform: Methanol: Triethylamine (7:2:1:2 v/v/v/v) **R_f:** **Finasteride:** 0.35 **Tamsulosin Hydrochloride:** 0.78 **Wavelength:** 230nm	[23]

		Method: RP-HPLC **Stationary phase:** C18 (250 x 4.6mm, 5µ) Phenomenex **Mobile phase:** Acetonitrile: Buffer adjusted to pH 3 (60:40 v/v) **Wavelength:** 230nm **Flow rate:** 0.8ml/min	
4.	Validated RP-HPLC and TLC methods for simultaneous estimation of tamsulosin hydrochloride and finasteride in combined dosage forms	**Method: HPTLC** **Stationary Phase:** silica gel 60 F_{254} **Mobile Phase:** g toluene/ methanol/ triethylamine (9: 1.5: 1 v/v/v) **R_f:** **Finasteride:** 0.65 **Tamsulosin Hydrochloride:** 0.36 **Wavelength:** 270nm **Method: RP-HPLC** **Stationary Phase:** A Phenomenex C18 column **Mobile Phase:** Methanol/0.02 mol L^{-1} ammonium acetate buffer/triethylamine (79.9: 20: 0.1 V/V/V) (pH 9.2) **Wavelength:** 235nm **Flow rate:** 1 mL/min **Method: Spectroscopic method** **Q- Absorbance Method:** **Solvent:** Ethanol 95% v/v: Distilled water (30:50 v/v) **Wavelength:** 217.7 nm **Linearity**: **Finasteride:** 5-30µg/ml **Tamsulosin Hydrochloride:**5-30µg/ml **R^2:** **Finasteride:** 0.35 **Tamsulosin Hydrochloride:**0.78 **% Recovery:** **Finasteride:**100.339% **Tamsulosin Hydrochloride:** 99.397%	[24]
5.	Simultaneous estimation of finasteride and tamsulosin hydrochloride by reverse phase HPLC in bulk and pharmaceutical dosage form	**Stationary Phase:** C18 (150mm x 4.6mm i.d., 5µm particle size) **Mobile Phase:** Buffer: Acetonitrile: Water (15:75:10 v/v) **Retention time:** **Finasteride:** 2.325 min **Tamsulosin Hydrochloride:** 4.296 min **Wavelength:** 245 nm **Flow rate:** 0.8 ml/min	[25]
6.	Simultaneous Estimation of Finasteride and Tamsulosin Hydrochloride in Combined Dosage Forms by RP-HPLC-	**Stationary Phase:** C18 column (150 x 4.6 mm, 5 µ) **Mobile Phase:** Methanol: Formic acid (0.02% v/v in water)	[26]

	PDA Method	**Retention time:** **Finasteride:** 2.7 min **Tamsulosin Hydrochloride:** 10.08 min **Wavelength:** 230 nm **Flow rate:** 1 mL/min	
7.	RP-HPLC Method for Simultaneous Estimation of Finasteride and Tamsulosin in Tablet Formulations	**Stationary Phase:** Spherisorb C-18 **Mobile Phase:** Methanol: 0.03mM phosphate buffer pH 3.5 (70:30 v/v) **Wavelength:** 210nm **Flow rate:** 1.0ml/min	[27]
8.	Simultaneous Determination of Finasteride and Tamsulosin in Combined Dosage Form by Using RP-HPLC Method	**Stationary Phase:** C18 column (150× 4.6 mm i.d, particle size of 5µ) **Mobile Phase:** 0.1% triethylamine (pH adjusted to 7.01 ±0.05 with 0.1% ortho phosphoric acid) and methanol (30:70% v/v) **Retention time:** **Finasteride:** 5.8 ±0.12 min **Tamsulosin:** 2.9± 0.14 min **Wavelength:** 220 nm **Flow rate:**0.7 ml/min	[28]
9.	RP HPLC method for the determination of finasteride and tamsulosin in bulk and pharmaceutical formulations	**Stationary Phase:** Hypersil ODS C18 Column 250 X 4.6 mm (particle size of 5µ) **Mobile Phase:** Acetonitrile: (0.05M) KH_2PO_4 buffer (45:55 v/v) **Retention time:** **Finasteride:** 3.59 min **Tamsulosin:** 6.051 min **Wavelength:** 240nm **Flow rate:** 1.8 ml /min	[29]
10.	Development and Validation of Analytical Method for Simultaneous Determination of Minoxidil and Finasteride in Pharmaceutical Dosage Form by RP-HPLC Method	**Stationary Phase:** ODS C18 column (25 cm × 4.6 mm, 5 µ particle size) **Mobile Phase:** Methanol: Water along with 0.5 % triethyl amine (TEA), pH 6.38 adjusted with ortho phosphoric acid (OPA) (70:30 v/v) **Retention time:** **Finasteride:** 4.661 min **Minoxidil:** 10.005 min **Wavelength:** 210 nm **Flow rate:**1ml/min	[30]
11.	Chromatographic Methods for Determination of Finasteride and Tamsulosin Hydrochloride and in Presence of Finasteride Degradation Product	**Stationary phase:** C18 column (300 mm × 3.9 mm; 10-µm particle size) **Mobile phase:** Acetonitrile - 0.04 M ortho-phosphoric acid (pH 3.5 ± 0.2 adjusted with triethylamine) (50:50, v/v) **Retention time:** **Finasteride:** 4.0 ± 0.2 & 5.0 ± 0.2 min **Tamsulosin Hydrochloride:** 9.0 ± 0.2	[31]

		min **Wavelength:** 215 nm **Flow rate:** 1 mL/min	
12.	Development and Validation of Ratio Derivative UV Spectrophotometry Method for Simultaneous Determination of Tamsulosin Hydrochloride and Finasteride in Combined Dosage Form	**Ratio Derivative Method:** **Solvent:** Methanol **Wavelength:** **Finasteride:** 253.94 nm **Tamsulosin Hydrochloride:** 235.92 nm **Linearity:** **Finasteride:** 10-60 µg/mL **Tamsulosin Hydrochloride:** 1.6-8.0 µg/mL **R^2:** **Finasteride:** 0.9963 **Tamsulosin Hydrochloride:** 0.9998 **% Recovery:** **Finasteride:**9 8.02-100.79 % **Tamsulosin Hydrochloride:** 98.05-100.62 %	[32]
13.	Simultaneous estimation of tamsulosin hydrochloride and finasteride in combined dosage form by UV spectroscopy method	**Solvent:** Methanol **Wavelength:** **Finasteride:** 240 nm **Tamsulosin Hydrochloride:** 279 nm **Linearity:** **Finasteride:** 10-60µg/mL **Tamsulosin Hydrochloride:** 10-60 µg/ml **R^2:** **Finasteride:** 0.9991 **Tamsulosin Hydrochloride:** 0.9995 **% Recovery:** **Finasteride:** 99.65 **Tamsulosin Hydrochloride:** 99.45	[33]
14.	Simultaneous determination of Finasteride and Tamsulosin in pharmaceutical preparations by ratio derivative spectroscopy	**Ratio derivative spectroscopic method** **Solvent:** Methanol **Wavelength:** **Finasteride:** 240.01 **Tamsulosin:** 229.91 **Linearity:** **Finasteride:** 2-10 µg/mL **Tamsulosin:** 25-125 µg/mL **R^2:** **Finasteride:** 0.998 **Tamsulosin:** 0.999 **LOD:** **Finasteride:** 0.21 µg/ml **Tamsulosin:** 0.19 µg/ml **LOQ:** **Finasteride:** 0.647 µg/ml **Tamsulosin:** 0.54 µg/ml	[34]

		% Recovery: **Finasteride:** 99.32 % to 100.59 % **Tamsulosin:** 99.87% to 101.23 %	
15.	UV Spectrophotometric Method for Simultaneous Determination of Tamsulosin and Finasteride in Combined Dosage Form	**Solvent:** Methanol **Wavelength:** **Finasteride:** 235 nm **Tamsulosin:** 225 nm **Linearity:** **Finasteride:** 12.5 - 100 µg/ml **Tamsulosin:**1- 10 µg/ml **R^2:** **Finasteride:** 0.9994 **Tamsulosin:** 0.9992 **% Recovery:** **Finasteride:** between 98.0-99.8% **Tamsulosin:** between 98.0-99.8%	[35]
16.	UV Spectrophotometric Method for Simultaneous Determination of Finasteride and Tamsulosin in Combined Dosage Form	**Solvent: Methanol** **Wavelength:** **Finasteride:** 219 nm **Tamsulosin:** 224nm **Linearity:** **Finasteride:** 12.5-62.5 µg/ml **Tamsulosin:**1-5 µg/ml **R^2:** **Finasteride:** 0.9981 **Tamsulosin:** 0.9989 **% Recovery:** **Finasteride:** 99.76 % **Tamsulosin:** 99.85 %	[36]

Literature review of tadalafil

- **Pharmacopeial methods for estimation of tadalafil**

Sr. No.	Drug/ Formulation	Method	Official in	Description	Ref. No.
1.	Bulk drug	HPLC	IP 2018	**Stationary phase:** A stainless steel column 25 cm x 4.6 mm packed with Silica gel AD for chiral separation. **Mobile phase:** Equal volumes of hexane and isopropyl alcohol. **Flow rate:** 0.75 ml/min **Wavelength:** 222 nm	[4]
2.	Bulk drug	HPLC	IP 2018	**Stationary phase:** A stainless steel column 25 cm x 4.6 mm packed with octadecylsilane bonded to porous silica (5 µm). **Mobile phase:** A. add 1.0 ml of trifluoro acetic acid to 1000ml water, B. acetonitrile.	[4]

				Flow rate: 1 ml/min **Wavelength:** 285 nm	
3.	Bulk drug	HPLC	IP 2018	**Stationary phase:** A stainless steel column 25 cm x 4.6 mm, packed octadecylsilane bonded to porous silica (5 µm). **Mobile phase:** A mixture of 45 volumes of acetonitrile and 55 volumes of solution A. **Flow rate:** 1.5 ml/min **Wavelength:** 285 nm	[4]
4.	Tablet	HPLC	IP 2018	**Stationary phase:** A stainless steel column 5 cm x 4.6 mm packed octadecylsilane bonded to porous silica (3.5 µm). **Mobile phase:** A mixture of equal volumes of methanol and water. **Flow rate:** 2 ml/min **Wavelength:** 225 nm	[4]
5.	Bulk drug	LC	USP 2019	**Stationary phase:** 4.6 mm × 25 cm; 5 µm packing L7 **Mobile Phase:** acetonitrile: solution A (Add 1 ml of trifluoroacetic acid to 1L of water (45:55) **Flow rate:**1.5 ml/min **Wavelength:** 285 nm **Column temperature:** 44 °C **Injection volume:** 20 µl	[10]
6.	Bulk drug	LC	USP 2019	**Stationary phase:** 4.6 mm × 25 cm; 5 µm packing L7 **Mobile Phase:** acetonitrile: solution A (Add 1 ml of trifluoroacetic acid to 1L of water (15.85, 95:5) **Flow rate:** 1.0 ml/min **Wavelength:** UV 285 nm **Column temperature:** 40 °C **Injection volume:** 20 µl	[10]
7.	Bulk drug	LC	USP 2019	**Stationary phase:** 4.6 mm × 25 cm; 10 µm packing L7 **Mobile Phase:** Hexenes: Isopropyl alcohol (50:50 v/v) **Flow rate:** 0.75 ml/min **Wavelength:** 222 nm **Column temperature:** 30 °C **Injection volume:** 10 µl	[10]
8.	Tablet	LC	USP 2019	**Stationary phase:** 4.6 mm × 25 cm; 3.5 µm packing L7 **Mobile Phase:** Acetonitrile:	[10]

				Water: Trifluoro acetic acid (35:65:0.1 v/v/v) **Flow rate:**1.0 ml/min **Wavelength:** UV 285 nm **Column temperature:** 35 °C **Injection volume:** 10 µl	
9.	Tablet	LC	USP 2019	**Stationary phase:** 4.6 mm × 25 cm; 3.5 µm packing L7 **Mobile Phase:** Methanol: Water (50:50 v/v) **Flow rate:** 2.0 ml/min **Wavelength:** UV 285 nm **Column temperature:** 40 °C **Injection volume:** 50 µl	[10]
10.	Oral solution	LC	USP 2019	**Stationary phase:** 4.6 mm × 25 cm; 5 µm packing L1 **Mobile Phase:** Acetonitrile: Solution A (10mM solution phosphate adjusted with phosphoric acid to a pH of 3.0 pass through a nylon filter of 0.45 µm pore size. **Flow rate:** 0.8 ml/min **Wavelength:** UV 220 nm **Column temperature:** 30 °C **Injection volume:** 25 µl	[10]

- **Reported method for estimation of tadalafil**

Sr. No.	Method	Description	Ref. No.
1.	High Performance Thin Layer Chromatographic Method for Determination of Tadalafil in Tablet Dosage Form	**Stationary Phase:** Silica gel 60 F_{254} TLC plate **Mobile Phase:** Chloroform: Methanol (9:1 v/v) **R_f:** 0.78 + 0.008 **Wavelength:** 285 nm	[37]
2.	Stability Indicating HPTLC Determination of Tadalafil Hydrochloride in Bulk Drug and Pharmaceutical Formulations	**Stationary Phase:** Pre-coated silica gel 60F_{254} aluminum plates **Mobile Phase:** Hexane: Isopropyl alcohol: Acetonitrile (5:4:1 v/v/v) **R_f:** 0.65 **Wavelength:** 285nm	[38]
3.	High Performance Liquid Chromatographic Method for Determination of Tadalafil in Tablets and Wastewater	**Stationary Phase:** SupelcoC18 column (25cm x 4.6 mm; 5 µm) **Mobile Phase:** Methanol: Water: Triethylamine (60:38:2 v/v/v) pH adjusted to 4.0 with dilute phosphoric acid **Retention time:** 3.6 min	[39]

		Wavelength: 220 nm **Flow rate:** 1.3 ml/min	
4.	High-performance liquid chromatography with diode array detection method for the simultaneous determination of seven selected phosphodiesterase-5 inhibitors and serotonin reuptake inhibitors used as male sexual enhancers	**Stationary Phase:** Waters C8 column (4.6 × 250 mm, 5 m) **Mobile Phase:** Phosphate buffer pH 3, acetonitrile and methanol in the ratio 60:33:7 v/v/v **Retention time:** 13.4 min **Wavelength:** 225 nm **Flow rate:** 1.2 mL/min	[40]
5.	Determination of tadalafil in pharmaceutical preparation by HPLC using monolithic silica column	**Stationary Phase:** Chromolith Performance RP-18e (100 mm × 4.6 mm, i.d.) **Mobile Phase:** Phosphate buffer (100 mM, pH 3.0)-Acetonitrile (80:20, v/v) **Wavelength:** 230 nm **Flow rate:** 5 ml/min	[41]
6.	Validation and Method Development of Tadalafil in Bulk and Tablet Dosage Form by RP-HPLC	**Stationary Phase:** Agilent Eclipse XDB C 18 column (150 mm × 4.6 mm, 5 µ) **Mobile Phase:** Buffer (potassium dihydrogen orthophosphate) and acetonitrile in the ration of 50:50 v/v **Retention time:** 3.181min **Wavelength:** 285 nm **Flow rate:** 1.2 ml/min	[42]
7.	RP-HPLC Method Development and Validation of Tadalafil in Tablet Dosage Form	**Stationary Phase:** Agilent eclipse C_{18} column (4.6 x 250mm, 5um) **Mobile Phase:** Phosphate buffer pH 4.0: Acetonitrile (50:50 v/v) **Retention time:** 6 min **Wavelength:** 284nm **Flow rate:** 1.0ml/min	[43]
8.	Rapid Resolution RP-HPLC-Dad Method for Simultaneous Determination of Sildenafil, Vardenafil, and Tadalafil in Pharmaceutical Preparations and Counterfeit Drugs	**Stationary Phase:** Agilent Zorbax SB C8 column (50 ×4.6 mm i.d., 1.8 lm particle size) **Mobile Phase:** 0.030M of ammonium formate (adjusted to pH 3.0 with formic acid) and acetonitrile in the ratio 70:30 v/v **Retention time:** 5.067 min **Wavelength:** 230 nm **Flow rate:** 1.3 ml/min	[44]
9.	Validation and stability indicating RP-HPLC method for the determination of tadalafil API in pharmaceutical formulations	**Stationary Phase:** Inertsil C18, (5 µ, 150 mm x 4.6 mm) **Mobile Phase:** Acetonitrile: Phosphate buffer (70:30 v/v, pH 7.0) **Retention time:** 2.88 min **Wavelength:** 260 nm	[45]

		Flow rate: 0.8 ml/min	
10.	Integrated Quality by Design (QbD) Approach for Stability Indicating RP-HPLC Method for the Estimation of Tadalafil Hydrochloride in Bulk Drug and Pharmaceutical Formulations	**Stationary Phase:** JASCO Crest Pack C18 (250mm×4.6mm, 5µm) **Mobile Phase:** Acetonitrile: Methanol (40:20 v/v) **Wavelength:** 285nm **Flow rate:** 1.0ml/min	[46]
11.	UV Spectrophotometric Method for the Estimation of Tadalafil in Bulk and Tablet Dosage form	**Solvent:** Methanol **Wavelength:** 284 nm **Linearity:** 2-20 mcg/ml	[47]
12.	A Stress Degradation Kinetic Study of Tadalafil Bulk and Tablet Dosage Form by UV Spectrophotometry	**Solvent:** Methanol **Wavelength:** 284 nm **Linearity:** 4-40µg/ml **R^2:**0.9994 **LOD:** 0.313µg/ml **LOQ:** 0.950µg/ml **% Recovery:** 97.06 - 99.56 %	[48]
13.	Estimation of Tadalafil Using Derivative Spectrophotometry in Bulk Material and in Pharmaceutical Formulation	**Solvent:** Dimethylfuran **Wavelength:** **Method A:** 297 nm **Method B**: 290.60–304.40 nm **Method C:** 284 nm **Method D:** 280.80–286.20 nm **Linearity:** **Method A & B:** 05–50 μg/mL **Method C & D:** 20–70 μg/mL **R^2:**> than 0.999 (For Method A, B, C & D)	[49]
14.	Estimation of Tadalafil in Bulk and in Formulation by UV-Visible Spectrophotometry	**Solvent:** Ethanol: Water (80:20 v/v) **Wavelength:** **Method A:** 284.5 nm **Method B:** 828 nm **Linearity:** **Method A:** 5-30 µg/ ml **Method B:** 2-10 µg/ ml **R^2:** **Method A:** 0.9999 **Method B:** 0.9999 **LOD:** **Method A:** 0.0799 **Method B:** 0.0322 **LOQ:** **Method A:** 0.2423 **Method B:** 0.0976 **Recovery:** **Method A:** 100.34 ± 1.363 % **Method B:** 100.04 ± 0.345 %	[50]

➢ **Reported methods for estimation of tadalafil with other drugs**

Sr. No.	Method	Description	Ref. No.
1.	Novel HPTLC densitometric methods for determination of tamsulosin HCl and tadalafil in their newly formulated dosage form: Comparative study and green profile assessment	**Stationary Phase:** HPTLC silica gel 60 F_{254} plates **Mobile Phase:** **Method 1:** Ethyl acetate: Toluene: Methanol: Ammonia (5:3:2:0.5 v/v) **Method 2:** Ethyl acetate: Ethanol: Ammonia (8:2:0.1 v/v) **R_f:** **Method 1:** **Tadalafil:** 0.60 **Tamsulosin:** 0.23 **Method 2:** **Tadalafil:** 0.65 **Tamsulosin:** 0.41 **Wavelength:** 280 nm	[51]
2.	A Validated Green HPTLC Method for Quantitative Determination of Dapoxetine Hydrochloride and Tadalafil in Bulk and Pharmaceutical Formulations	**Stationary Phase:** Silica gel HPTLC F_{254} **Mobile Phase:** Ethanol: Ethyl acetate (1:9 v/v) **R_f:** **Tadalafil:** 0.75 ± 0.01 **Depoxetin Hydrochloride:** 0.4 ± 0.01 **Wavelength:** 222 nm	[52]
3.	A Validated Green HPTLC Method for Quantitative Determination of Dapoxetine Hydrochloride and Tadalafil in Bulk and Pharmaceutical Formulations	**Stationary Phase:** Hypersil BDS C8, 250 × 4.6 mm column **Mobile Phase:** Buffer (adjusted to pH 6.8): Acetonitrile (55:45 v/v) **Retention time:** **Tadalafil:** 4.473 min **Depoxetin Hydrochloride:** 5.836 min **Wavelength:** 254 nm **Flow rate:** 1.0 ml/min	[53]
4.	A Novel Chromatographic Method with Flouroscence Detection for Quantitation of Tadalafil and Depoxetin Hydrochloride in Pharmaceutical Dosage Form and Human Plasma	**Stationary Phase:** Eclipse C18 Column (150 mm×4.6 mm, 5 µm) **Mobile Phase:** Acetonitrile: 0.15% triethylamine (40:40 v/v) (ph:4) **Retention time:** **Tadalafil:** 6.642 min **Depoxetin Hydrochloride:** 4.819 min **Wavelength:** 236nm **Flow rate:** 1.0 ml/min	[54]
5.	A Novel Validated Chromatographic Method for Tadalafil and Dapoxetine	**Stationary Phase:** Kromasil C18 column (250 mm X 4.6 mm, 5 µm) **Mobile Phase:** ACN: Buffer (10:90 v/v, pH 5.5 adjusted with ortho phosphoric	[55]

	Hydrochloride in Combined Pharmaceutical Formulations	acid and diethyl ether) **Retention time:** **Tadalafil:** 2.806 min **Depoxetin Hydrochloride:** 5.965 min **Wavelength:** 290 nm **Flow rate:** 1.0 ml/min	
6.	A combined approach of green chemistry and Quality-by-Design for sustainable and robust analysis of two newly introduced pharmaceutical formulations treating benign prostate hyperplasia	**Stationary Phase:** Monolithic-based C18 column **Mobile Phase:** Ethanol and phosphate buffer (pH 4.0) in a ratio of 40:60 v/v **Wavelength:** 210 nm **Flow rate:** 2.3 ml/min	[56]
7.	Implementing Analytical Quality by Design (AQbD) Approach for Simultaneous Estimation of Tadalafil and Macitentan by RP-HPLC Method	**Stationary Phase:** Phenomexgemini C18 (150 mm, 4.6 mm, 5µm) **Mobile Phase:** Methanol: 10 mM ammonium formate (74.1: 25.9 % v/v) (pH: 6.8) **Retention time:** **Tadalafil:** 4.0 ± 0.1 min **Mecitentan:** 7.2 ± 0.1 min **Wavelength:** 260 nm **Flow rate:** 0.94 ml/ min	[57]
8.	A Novel Analytical Method for Simultaneous Quantification of Silodosin and Tadalafil by RP-HPLC	**Stationary Phase:** Supelco C8 (150mmx4.6mm, 5µm) **Mobile Phase:** Potassium phosphate dibasic buffer pH (4.3) and acetonitrile in the ratio of (70:30 v/v) **Wavelength:** 232 nm **Flow rate:** 1.0 ml/min	[58]
9.	Stability-Indicating RP-HPLC Method for the Determination of Ambrisentan and Tadalafil in Pharmaceutical Dosage Form	**Stationary Phase:** Hypersil GOLD C18 column (150 mm × 4.6 mm internal diameter, 5 µm particle size) **Mobile Phase:** Methanol: Water: Acetonitrile (40:40:20 v/v) **Retention time:** **Tadalafil:** 7.10 min **Ambriesentan:** 2.80 min **Wavelength:** 260 nm **Flow rate:** 0.5 ml/min	[59]
10.	Development and Validation of a New Stability Indicating RP HPLC Method for Simultaneous Estimation of Tadalafil and Depoxetin	**Stationary Phase:** Xterra C18 column (250mm x 4.6mm x5µm particle size) **Mobile Phase:** 0.1% OPA (pH 2.8) and Methanol (45:55v/v) **Retention time:** **Tadalafil:** 2.78 min **Depoxetin:** 3.71 min **Wavelength:** 229mn	[60]

		Flow rate: 1ml/min	
11.	Development and Validation of Stability Indicating RP-HPLC Method for Estimation of Finasteride and Tadalafil Hydrochloride in Solid Dosage Form	**Stationary Phase:** Water Symmetry C-18 (150 x 4.6 mm) **Mobile Phase:** Buffer: Acetonitrile (65:35 v/v) **Retention time:** **Tadalafil:** 10.08 min **Depoxetin Hydrochloride:** 4.45 min **Wavelength:** 285 nm **Flow rate:** 1.0 ml/min	[61]
12.	Smart spectrophotometric assessment of tamsulosin hydrochloride and tadalafil in their new pharmaceutical formulation for treatment of benign prostatic hyperplasia and erectile dysfunction	**Solvent:** Methanol **Linearity:** **Tadalafil:** 2.0-55.0 µg/mL **Tamsulosin:** 2.0-40.0 µg/mL **First derivative method** **Wavelength:** **Tadalafil:** 245.0 & 279.0 nm **Tamsulosin:** 284.0 nm **Ratio difference method:** **λmax:** **Tadalafil:** Between 244.0 and 292.0 nm **Tamsulosin:** Between 275.0 and 244.0nm **Derivative ratio method** **Wavelength:** **Tadalafil:** 241.0 & 248.0 nm **Tamsulosin:** 236.0, 254.0 & 283.0 nm **Mean centering of ratio spectra method** **Wavelength:** 244 nm	[62]
13.	Quantitative determination of Dapoxetine Hydrochloride and Tadalafil using different validated spectrophotometric methods	**Solvent:** Methanol **Wavelength:** **Method A: First derivative spectra** **Tadalafil:** 230 nm **Depoxetin Hydrochloride:** 322.4 nm **Method B: Area under curve (AUC)** **Tadalafil:** 242-254 nm **Depoxetin Hydrochloride:** 228-240 nm **Linearity:** **Tadalafil:** 3-30 mg/ml **Depoxetin Hydrochloride:**2-15 mg/ml	[63]
14.	A synchronous spectrofluorometric technique for simultaneous detection of alfuzosin and tadalafil: applied to tablets and spiked biological samples	**Solvent:** Ethanol **Wavelength:** **Tadalafil:** 293 nm **Alfuzosin:** 366 nm **Linearity:** **Tadalafil:**10.0–100.0 ng/ml **Alfuzosin:** 5.0–90.0 ng/ml **R^2:**	[64]

		Tadalafil: 0.9999 **Alfuzosin:** 0.9999 **LOD:** **Tadalafil:**1.52 **Alfuzosin:** 0.72 **LOQ:** **Tadalafil:** 4.61 **Alfuzosin:** 2.18 **% Recovery:** **Tadalafil:** 99.73% **Alfuzosin:** 100.44%	

Literature review of Finasteride and Tadalafil in combined dosage form

Sr. No.	Method	Description	Ref. No.
1.	Simultaneous spectrophotometric determination of finasteride and tadalafil in recently FDA approved Entadf™ capsules	**Derivative Spectroscopic Method:** **Second derivative with zero crossing method (^{2}D)** **Solvent:** Methanol **Wavelength:** **Finasteride:** 230.80 nm **Tadalafil:** 292nm **Linearity:** **Finasteride:** 10-140 µg/ml **Tadalafil:** 3- 40 µg/ml **R^2:** **Finasteride:** 0.9997 **Tadalafil:** 0.9996 **LOD:** **Finasteride:** 2.406 **Tadalafil:** 0.876 **LOQ:** **Finasteride:** 7.292 **Tadalafil:** 2.654 **% Recovery:** **Finasteride:** 99.37 % **Tadalafil:** 99.17 % **First derivative of ratio spectra method (^{1}DD)** **Solvent:** Methanol **Wavelength:** **Finasteride:** 218.80 nm **Tadalafil:** 289.60 nm **Linearity:** **Finasteride:** 10–140 µg/ml **Tadalafil:** 3–40 µg/ml **R^2:** **Finasteride:** 0.9998 **Tadalafil:** 0.9996	[65]

		LOD: **Finasteride:** 2.229 **Tadalafil:** 0.815 **LOQ:** **Finasteride:** 6.755 **Tadalafil:** 2.470 **% Recovery:** **Finasteride:** 99.74 % **Tadalafil:** 99.56 %	
2.	Selective and rapid determination of tadalafil and finasteride using solid phase extraction by high performance liquid chromatography and tandem mass spectrometry	**Stationary phase:** Zorbax Eclipse C18 column (50 × 4.6 mm, 5 µm) **Mobile phase:** Mixture of 4 mM ammonium formate (pH 4.0): acetonitrile: methanol 20:45:35 (v/v/v) was filtered through a 0.45 µm membrane filter **Retention time:** **Finasteride:** 1.63 min **Tadalafil:** 1.41 min **Linearity:** **Finasteride:** 0.2–30.0 ng/ml **Tadalafil:** 5–800 ng/ml **Flow rate:** 0.7 ml/min **Wavelength:** 210 nm. **Injection Volume:** 20 µl **Column temperature:** 35 °C	[65]

CONCLUSION

This review present general knowledge about Finasteride and Tadalafil and also general introduction about analytical method validation. This review presents analytical methods applied for the determination of Finasteride and Tadalafil for individual drugs or in combination with other drugs as well as this literature review includes reported pharmacopeial methods for both the drugs. At present, various analytical methods like Spectrophotometry, RP-HPLC, HPTLC, are available for the simultaneous estimation of Finasteride with combination of drugs like Minoxidil, Tamsulosin, Depoxetin etc. Similarly, for estimation of Tadalafil, Various analytical methods are available for the simultaneous estimation with Tamsulosin, Depoxetin Mecitentan, Silodosin etc. From this literature review, it can be concluded that there is only one UV Spectrophotometry method and one LC/MS method till date has been reported. This literature review will be helpful for further research on both of this drug and their combination for future analytical studies. This can be used as reference for further method development and validation in future.

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