Review Article

Hacettepe University Journal of the Faculty of Pharmacy
Volume 42 / Number 2 / June 2022 / pp. 134-147

Characteristics and Analytical Methods of Novel PDE5 Inhibitor Avanafil: An Update

Alankar SHRİVASTAVA
ORCID: 0000-0002-2498-9875

KIET Group of Institutions (KIET School of Pharmacy), Ghaziabad (UP), India

Corresponding author:
Alankar SHRIVASTAVA
KIET Group of Institutions (KIET School of Pharmacy), Ghaziabad (UP), India
E mail: alankar.shrivastava@kiet.edu

Doi: 10.52794/hujpharm.1017182

ABSTRACT

Erectile dysfunction (ED) affects middle and older age and occurrence increasing with age of men. PDE5 inhibitors are commonly used for the treatment of ED. The incidences of adulteration of PDE5 inhibitors with herbal aphrodisiacs is evident in almost every part of world. Avanafil (AVA) is novel PDE5 inhibitor with advantage of fast action. The literatures and various analytical methods presented in this review was obtained by searching various databases like pubmed, medline, eurekaselect, sciencedirect, wiley and taylor and francis journals. The services of google scholar were also utilized for searching some literature. The literature search was performed to summarize characteristics, synthesis and analytical methods for determination of AVA. Different spectrophotometric methods, chromatographic and ion mobility mass spectrometric methods are available in the method. The possibility for the development of green analytical method is also discussed. The various analytical methods and characteristics for AVA analysis are described. The methods for determination of AVA as adulterants and in pharmaceutical formulations including stability indicating methods.

Keywords: Spectrophotometry, chromatography, erectile dysfunction, avanafil, adulteration analysis

Received date : 01.10.2021
Accepted date : 27.01.2022

Introduction

The Fourth International Consultation on Sexual Medicine defines erectile dysfunction (ED) as "*the consistent or recurrent inability to attain and/or maintain penile erection sufficient for sexual satisfaction*".[1] ED is perhaps the most widely recognized condition influencing moderately aged and more aged men. The reality is that ED is a normal aspect of growing older, and its prevalence rises with age. It is estimated that 50% of men in their fifties, 60% of men in their sixties, and 70% of men in their seventies had ED.[2]

ED is a prevalent clinical condition that primarily affects males over the age of 40. Several common lifestyle factors, such as obesity, little or no physical activity, and lower urinary tract symptoms, have been associated to the development of erectile dysfunction. The conventional causes of ED are hypertension and diabetes.[3] Nerve impulses release neurotransmitters from cavernous nerve terminals and relaxing substances from endothelial cells during sexual excitement, resulting in vascular smooth muscle relaxation and increased blood flow to the penis.[4]

ED can be caused by common medications such antihypertensives, nonsteroidal anti-inflammatory drugs, and antacids.[5] Phosphodiesterase 5 (PDE5) is a well-studied phosphodiesterase (PDE) that selectively targets cyclic guanosine monophosphate (cGMP), which is generally produced by NO-mediated activation of the soluble guanylyl cyclase.[6] Phosphodiesterase type 5 (PDE5) inhibitors are powerful oral medicines for ED and have become quite possibly the most generally recommended medication worldwide.[7][8]

Around the world, the utilization of dietary enhancements for the upgrade of sexual execution is normal. Consumers are by and large enamored with these items since they frequently need to stay away from drugs, liking "normal" than "synthetic" solutions.[9]

The US-FDA, from 2007 to 2014, revealed 572 instances of supplement defilements in the country, mostly items for sexual improvement (41.6%). Information from the European showed 929 debasements during a similar period, more than 40% because of unauthorized ingredients or undeclared medicines.[10] The adulterations commonly include adding unlabelled medications into relating natural enhancements, e.g., sedative, sexual enhancer and weight reduction items, planning to make them show quicker and more critical drug impacts and increment the deals of the items.[11]

AVA specifically inhibites phosphodiesterase type 5 (PDE5) and is utilized as treatment of ED which intervenes the breakdown of cGMP, initiating smooth muscle relaxation in the corpus cavernosum of the penis.[12] It was approved by US FDA in 27th April, 2012 indicated for the treatment of erectile dysfunction.[13] Orally active PDE5 inhibitors like sildenafil, tadalafil and vardenafil, are at present the best option treatment choices for ED. Be that as it may, a critical number of patients stay disappointed with the accessible treatments due an absence of adequacy or distress emerging from adverse events. In phase II and phase III trails, AVA has demonstrated improved efficacy, tolerability and selectivity for PDE5, is rapidly absorbed after oral administration with a fast onset of action and a plasma half-life that is practically identical to sildenfil and vardenafil.[14]

The IUPAC name is "(4-[(3-chloro-4-methoxybenzyl) amino]-2-[2-(hydroxymethyl)-1-pyrrolidinyl]- *N*-(2-pyrimidinylmethyl)-5-pyrimidinecarboxamide; (*S*)-2-(2-hydroxymethyl-1-pyrrolidinyl)-4- (3-chloro-4- methoxybenzylamino)-5-[(2-pyrimidinylmethyl) carbamoyl] pyrimidine)" (Figure 1) is a pyrimidine derivative (MW=483.95), and exists as a solitary enantiomer with *S* stereochemistry. In its pure form, it exists as a white translucent powder, which is negligibly dissolvable in water and modestly dissolvable in organic solvent. Solubility testing at various pH values revealed uncovered expanded solvency in acidic cushions (~pH 4) and diminished solvency in neutral and alkaline buffers.[15] It has higher selectivity (120x) against PDE6 than sildenafil (16x) and vardenafil (21x), as well as much higher selectivity (>10 000x) against PDE1 compared with sildenafil (380x) and vardenafil (1000x).[16]

The literatures and various analytical methods presented in this review was obtained by searching various databases like Pub med, Medline, Eurekaselect, Science direct, Wiley and Taylor and Francis journals. The services of Google Scholar were also utilized for searching some literature.

Synthesis

The latest patent of Chongqing Aoshe Biochemical Co ltd describes the novel method for the preparati-

ISSN: 2458 - 8806

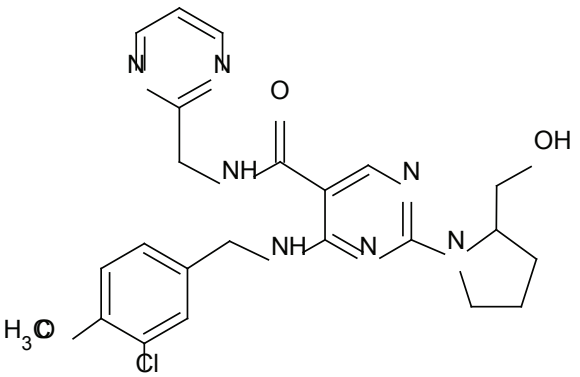

Figure 1: Chemical structure of Avanafil

on of AVA. The reaction of aryl boronic acid (containing a certain amount of aryl boronic acid anhydride) with carboxylic acid can generate monoacyloxy boronic acid active intermediates (which can be regarded as mixed acid anhydrides of carboxylic acid and aryl boronic acid). Intramolecular hydrogen bonds and electron deficient boron in reactive intermediates can be conclusive to the nucleophilic attacks of amines to activate the carboxyl carbon.[17] The scheme is presented in Figure 2.

Mechanism of action

There are 21 different PDE genes classified into 11 families (PDE 1 to 11) identified to date based on enzymatic properties, substrate specificity, sequence homogeneity, affectability to endogenous controllers and inhibitors.[18][19] PDE type 5 (PDE5) is the dominating type of the enzyme in the male penile smooth muscle of the corpora cavernosa.[20]

Thus, PDE5 enzyme should be target for drugs for treatment of ED in men. The other developed PDE5 inhibitors like Sildenafil, Tadalafil and Vardenafil are not selective and may also inhibit other PDE isoforms (PDE1, PDE6 and PDE11). The inhibition of these enzymes is reason of causing adverse effects like visual disturbance, loss of hearing ability and dyspepsia.[21-23]

The AVA is proved to be providing superior clinical efficacy and safety because of its specificity towards PDE5 blockage action. The reason behind this property is its unique structure which includes aryl halide (-Cl) rather than nucleotide (purine base-sugar-phosphodiester bonds) forming backbone of earlier discovered first line drugs for ED.[24]

In the recent study of Hsieh *et al* (2020)[25], the structure of PDE5-AVA complex was determined at 1.9 Å resolution. The PDE5 inhibitors are known to be act by competing with cGMP for binding to the active site. The structure of avanafil does not resembles guanine but because of H-bond formation between 5-carboxamide oxygen of the central pyrimidine-5-carboxamide and 4-alkyl amine, it starts mimicking the action of guanine. The bond was supported by other forces for further stabilization like other covalent connections, AVA-explicit H-bonding, van der Waals forces (with adjacent hydrophobic residues), interactions of π-π bonds, water mediated and direct H-bonds. The important highlights of this paper is presented under Figure 3.

Pharmacokinetics and Pharmacodynamics

AVA is having fast onset of action (15 mins) and absorption is delayed due to high fat food intake. The maximum duration of action is 6 hrs with half life ($T_{1/2}$) 3 to 5 hours. The shorter onset of action is unique property of avanafil compared with other PDE inhibitors.[26]

Hepatic metabolism is known mechanism for clearance of AVA, fundamentally by the cytochrome P450 isoenzyme CYP3A4 and, less significantly, by the CYP2C isoenzyme. Overall, there are 16 different identified metabolites but major portion consists M4- "4-((3-chloro-4-methoxybenzyl) amino)-2-((2S,4R)-4-hydroxy-2-(hydroxymethyl) pyrrolidin-1-yl)-N-(pyrimidin-2-ylmethyl)pyrimidine-5-carboxamide" and M16 – "4-((4-((3-chloro-4-methoxybenzyl)amino)-5-((pyrimidin-2-ylmethyl)carbamoyl)pyrimidin-2-yl)amino)-5-hydroxypentanoic acid". The former constitutes 23% plasma concentration of that of AVA and represents 4% of absolute pharmacologic action. The later one constitutes 29% plasma concentration of that of avanafil and inert towards PDE interaction. AVA is primarily excreted in feces (62%) and some portion in urine (21%).[27] M16 is obtained from the pyrrolidine ring α-carbon oxidation to carbinolamine, followed by ALDH mediated oxidation of its tautomeric aldehyde (ring opened) form (Figure 4).[28]

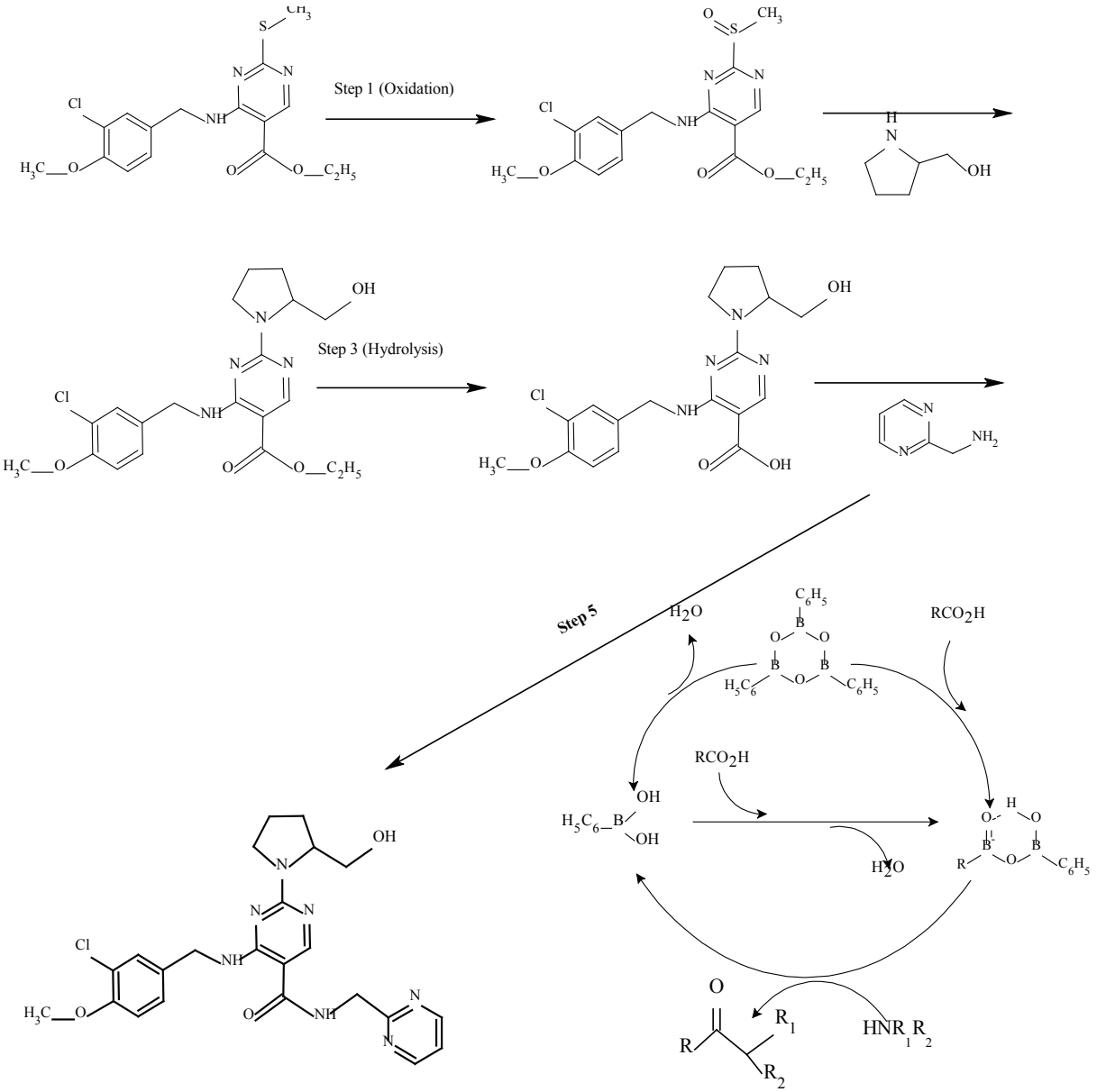

Figure 2: Scheme of synthesis of Avanafil

Drug interactions and contraindications

Dose adjustments is not required in renal and hepatic impairment in mild to moderate conditions. The utilization of both ketoconazole 400 mg every day and ritonavir 600 mg twice day by day has been displayed to build AVA's AUC concentration by 13-fold. As suggested by the producer, the avanafil portion ought not surpass 50 mg/day in patients who are likewise taking moderate CYP3A4 inhibitors (e.g. itraconazole, clarithromycin and ritonavir). AVA ought not be utilized in patients taking strong CYP3A4 inhibitors.[29]

There may be reduced clearance in people using fluoxetine. The actions of drugs mimicking the concentration of guanosine monophosphate (GMP) e.g. glyceryl trinitrate may be increased by AVA because of pharmacodynamic interactions and may cause severe hypotension. With antihypertensives, alcohol (additive effect) and alpha blockers may result vasodilatory action. AVA is not recommended in patients with cardiovascular disease.[30]

ISSN: 2458 - 8806

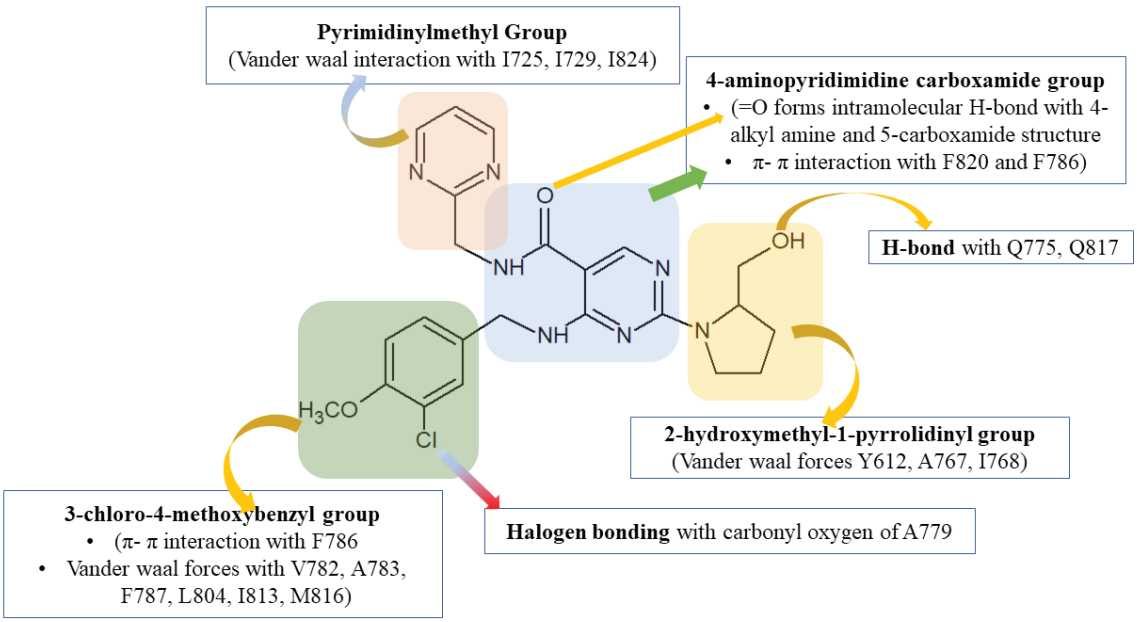

Figure 3: Mechanism of action of avanafil

AVA should be cautiously administered with rosiglitazone, omeprazole and desipramine because of risk of increase in plasma levels. Possible increase in hypotensive effect of riociguat, potentiate antiaggregatory effect of sodium nitroprusside and should be avoided in the risk of hypersensitive reactions with avanafil or any of its components.[31]

Analytical Methods

It is all around acknowledged that an estimation of an amount of interest ought to be introduced as the result of a mathematical worth and a unit.[32] In the upcoming section, different analytical methods reported are presented.

Spectrophotometric methods

Based on the Beer-Lambert law, spectrophotometric methods can estimate concentration of analyte can be estimated by comparing incident light with transmitted light through a solution.[33] The summary of spectrophotometric methods for the determination of AVA is presented in Table 1.

Chromatography methods

Reversed phase chromatography (RPC) is a usually utilized scientific strategy in the biotech and drug industries.[41] Computer-assisted strategies can uphold a portion of these choice advances limiting the time needed to fabricate a strong technique.[42] Planar chromatography is used over the years in the analysis of drugs, forensic analysis, dyes, pesticides, herbal drug analysis, plant toxins etc.[43] The summary of chromatographic methods is presented in Table 2.

.[42] Planar chromatography is used over the years in the analysis of drugs, forensic analysis, dyes, pesticides, herbal drug analysis, plant toxins etc.[43] The summary of chromatography methods is presented under Table 2.

Others

In the ion mobility spectrometry technique, the organic molecules are mobilized by electric field against the countercurrent of neutral drift gas after their ionization. Before reaching to the detector, ions interacts with the drift gas several times which reduces the speed. The ions are accelerated after each such interaction by the applied filed. When there is alternate repetition of this acceleration and interaction, the average ion velocity becomes constant

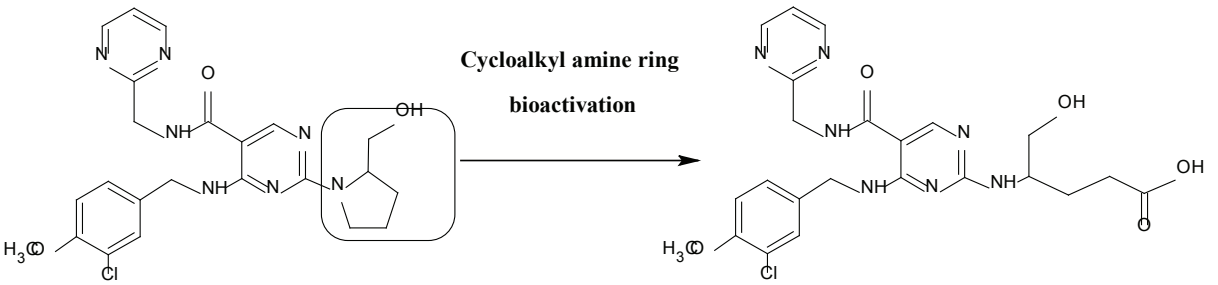

Figure 4: Conversion of M16 metabolite from Avanafil

which depends on cross section, charge and mass of ion. The downfield detector identifies the ions on the basis of their arrival times.[60] Mans *et al*. [61] determined three adulterants (acetildenafils, sildenafils and avanafil) in herbal supplement matrices using this technique and suggested as rapid toll for such analysis.

Discussion

AVA has a high proportion of inhibiting PDE5 as contrasted and other PDE subtypes taking into account the medication to be utilized for ED while limiting antagonistic effects.[62] AVA has an extremely fast beginning of activity in light of its quick absorption, which permits it to arrive at maximum circulating concentration in around 30 to 45 minutes.[63]

Analysis in food supplement and as adulterant

The PDE5 inhibitors are also reported to be found as adulterants in herbal aphrodisiacs and food supplements because of their action and AVA is also no exception. There are methods reported for determinations in such matrices. Within just three years of its approval by FDA, a Korean study published by Jeong *et al* [64] described analysis of various PDE5 including AVA in some food supplements. The another study [65] published in Chinese language related to detection in health foods. The LOD reported in former is 0.10 and latter study reported 0.21 µg/kg. The Korean study describes the analysis of AVA in complex samples along with various other PDE5 inhibitors[64] but latter describes the determination with Flibanserin (serotonin receptor 2A antagonist) [65].

The UPLC with MS/MS detection method was developed by Wang *et al* [49] for simultaneous determination of twenty three adulterated aphrodisiacs in health foods and Chinese traditional patent medicines. However, adulteration of AVA was not found in any investigated samples but method can be used for determination as adulterants.

The determination in illicit erectile dysfunction medications and human urine is also available in literature. [52] Results got uncovered the presence of significant degrees of sildenafil in samples, but can be extended for determination of AVA for same purpose.

Determination in pharmaceutical formulations

Spectrophotometry methods are more popular because of its speed and cost effectiveness analysis. Most of the drugs have chromophores in structure showing absorbances when scanned under UV light. This phenomenon is utilized for quantification of analytes. To solve the problems of overlapping absorbances higher derivative spectrums are also used. In this case also, apart from simple UV spectrophotometry method [34][37], derivative spectrophotometry was used for analysis. One more approach is to use spectrofluorometry to increase the selectivity in analysis compared with conventional technique. The problem of spectrum overlapping can be further overcome by using derivative fluorescence spectrums. This approach is used in recent publication [41] for simultaneous analysis of AVA and acidic degradation product that shows inherent fluorescence at 370 and 407 nm, after excitation at 268 and 271 nm.

The scientific literatures are full of HPLC methods developed for probably every possible analyte. One important reason is easy separation of various components of sample because of different affinity towards stationary phase.[66]

In the case of AVA, various chromatography methods were developed including determination in formulations including some combinations and related

ISSN: 2458 - 8806

substances. There is not an official method found for analysis.

Stability indicating methods

The evaluation of the chemical stability investigations of small molecule pharmaceuticals depends principally on the availability of a chromatographic or other separation assay fit for isolating and evaluating degradation products and major impurities.[67]

There are some publications of stability indicating method determinations. The stability indicating methods are aimed to establish storage conditions of drugs and identify and separate degradants in the presence of parent molecule.

The gradient stability indicating HPLC method developed by Kumar *et al*.[46] This method separates the degradation products from drug peak. Another method separates total three peaks of two related substances and degradation product from avanafil. The recent publication in this regard is isocratic HPLC-PDA method using QbD approach separating 16 degradation product peaks (in solid and solution both). The overall degradation of avanafil in stress conditions was found in order of sunlight > moisture > temperature.

Green analytical chemistry

The Green Analytical Chemistry concept started appearing in the literature from last decade of 20th century.[68] Since, AVA is reported to be Class I or Class II Biopharmaceutics Classification System (BCS)[69], this may be the reason that its metabolites are excreted mainly in the feces (approx 62% of administered oral dose).[70]

Thus, approach to increase the solubility of drug in water can be utilized for development of green analytical method. Since, avanafil is available in crystalline structure, reducing the size of particles to nanoscale may increase the solubility and then suitable analytical method can be developed and validated. This may also reduce the solvent cutoff especially helpful in development of more specific spectrophotometry method. Other than this, cosolvency, pH adjustment, surfactant addition, and complexation are the most widely recognized drug approaches for solubilising drug competitors with low aqueous solubility.[71]

LC-MS Methods

In the literature survey, eleven methods [44,49-55,57-59] found to be published based on LC-MS techniques for different matrices. Coupling of MS to chromatographic techniques has always been attractive due to the sensitive and exceptionally explicit nature of MS contrasted with other chromatographic detectors[72,73,74]. The relative sensitivity of these methods compared with other methods, particularly spectrophotometry methods is more, making them suitable for bioanalytical determinations. As already discussed, there is always probability of misuse of AVA mixing unethically with food or herbal medications to impart aphrodisiac activity. The LC-MS methods have potential applications is such cases which is also reflecting in this case (Refer Table 2).

Conclusion

Avanafil with advantage of rapid onset of action is having edge over other available PDE5 inhibitors. A recent survey also shown that there is remarkable increase in the sales of these drugs during covid pandemic.[75] This class of drugs have given option to overshine conventional medications like ayurvedic and herbal aphrodisiacs and created enormous benefits for their producers. This is obvious reason for adulteration of PDE5 inhibitors in such formulations. The critical review describes characteristics and synthesis of drug in the initial portions, followed by analytical methods in next section. The spectrophotometry and chromatography methods were given under Table 1 and 2 respectively. The aspects of future development in context to development of new green analytical methods were also discussed.

Acknowledgement

The authors acknowledge support given by KIET Group of Institutions, Ghaziabad for giving support for study.

Table 1. Summary of spectrophotometry methods

Method	Wavelength	Solvent	Calibration curve	LOD	LOQ	Application	Reference
First order derivative and dual wavelength method	210 nm, 206.80 nm and 214.80 nm	0.1N HCL	1-8 µg/ml, 1-6 µg/ml	0.03 µg/ml	0.12 µg/ml	Simultaneous estimation with Dapoxetine hydrochloride in tablets	[34]
Spectrofluorimetric	λ_{ex} 367 and λ_{em} 314 nm	Methanol	200–1000 ng/ml	7.32 ng/ml	22.18 ng/ml	In bulk and tablets	[35]
Chemometric-assisted methods (CLS, PLS, PCR, and NPLS)	226–328 nm, 51 wavelength points	ACN–water (50 + 50, v/v)	1–9 µg/mL	0.638, 0.598, 0.609, 0.612 µg/mL	1.932, 1.813, 1.846, 1.854 µg/mL	With dapoxetine (DP) in combination	[36]
Dual wavelength method	267 and 292 nm	Methanol	0.05-0.5 mg/mL	-	-	With its degradation products and in pharmaceutical formulation	[37]
First derivative (^{1}D) method	261 nm						
Derivative ratio (^{1}DR) method	275.6, 305.4 and 329 nm						
Ratio difference (RD) method	266 and 250 nm						
Native & synchronous fluorescence spectroscopy	λ_{ex}290 nm , λ_{em}314 nm, 267 nm (Δλ 90 nm)	Methanol	0.5 -16 µg/mL, 0.5-16 µg/mL	75 and 225 ng/mL	64 and 192 ng/mL	Simultaneous determination with dapoxetine hydrochloride and spiked plasma	[38]
Spectrofluorimetric	λ_{em} = 396 nm, (Δλ = 70 nm)	Britton-Robinson buffer (pH 4) and methanol	50–1800 ng mL^{-1}	12.93 ng mL^{-1}	42.67 ng mL^{-1}	Pure, tablets and spiked human plasma	[39]
Spectrofluorimetric	AVA λ_{em} 370, λ_{ex} 268, acid degradation product λ_{em} 407, λ_{ex} 271	Methanol	0.5–18 µg	0.01 µg/mL2	0.04 µg/mL	With acid-induced degradation product	[40]

ISSN: 2458 - 8806

Table 2. Summary of chromatography methods

Method	Detector	Column/ Stationary phase	Mobile Phase	Linear Range	LOD	LOQ	Matrix	Reference
HPLC	MS-MS	C_{18}	10 mM NH_4HCO_2 (pH 2.5) and ACN (v/v, 65:35)	1-250 ng/ ml	0.25 ng/ml	1 ng/ml	Plasma	[44]
HPLC	Fluorescence (236/370nm)	C_{18}	ACN: 0.15% triethylamine (40: 60, v/v) at pH=4.0	0.05- 40 µg/ml	0.043 µg/ ml	0.1306 µg/ml	Bulk powder, tablets and spiked human plasma	[45]
HPLC	UV (245 nm)	ODS	Gradient: A, 0.1% CF_3CO_2H and triethylamine in water, and B, H_2O, ACN ratio 20:80 (v/v)	0.1037 (LOQ) to 0.75 µg/ mL	0.0348 µg/ ml	0.1037 µg/ml	Degradation Products and Process-Related Impurities	[46]
HPLC	PAD (230 nm)	C_{18}	0.1 M NH_4HCO_2 buffer pH 2.5, MeOH, ACN with ratios (20:40:40)	10- 1000 µg/mL	2 ng/ml	10 ng/ml	Stability indicating	[47]
HPLC	UV (239 nm)	C_{18}	10 mM NH_4HCO_2 buffer–ACN (60 + 40, v/v), pH 3.7	10–150 µg/mL	0.0233 µg/ mL	0.0707 µg/mL	With Dapoxetine	[36]
HPLC	UV (245 nm)	C_{18}	A: pH 4.2 buffer: MeOH, B: ACN Ratio (A:B) was 90:10	0.048 (LOQ) to 70 µg/ml	0.0148 µg/ ml	0.048 µg/m	Related substances	[48]
HPLC	MS-MS	C_{18}	ACN + 0.1% CH_3COOH (containing 20 mmol·L^{-1} ammonium acetate)(60:40)	2-20 ng/ ml	0.21 µg/ ml	-	Determination of drug illegally added in food	[49]
UPLC	Q-TOF MS	C_{18}	A: 5 mmol/ L NH_4HCO_2 (pH 3.4 with acetic acid), and B: ACN.	0.05-10 µg/ml	0.02 µg/ ml	0.05 µg/ ml	Simultaneous with 23 illegal aphrodisiac in health foods and Chinese traditional patent medicines	[50]
HPLC	PDA (247 nm)	C_{18}	ACN:DMSO mixture (94:6 v/v)	0.5–20 µg/ mL	0.072 µg/ mL	0.217 µg/mL	Stability indicating and determination in tablets	[51]
HPLC	MS-MS	C_{18}	0.1% HCOOH in H_2O and 0.1% HCOOH in ACN (75:25 v/v)	150–6000 ng/mL	1.17 ng/ mL	3.55 ng/ mL		
HPLC	TOF-MS/MS	C_{18}	A: 10 mM ammonium formate and formic acid (pH 4.60), B: 0.10% (v/v) HCOOH in ACN	5.0-1000 ng/g	1.63 ng/g	5.43 ng/g	Illicit ED medications and human urine	[52]
HPTLC	UV (230 nm)	Silica gel 60 F_{254}	$CHCl_3$: toluene: MeOH: conc. NH_3 (6:5:3:0.1, v/v)	0.5-5 µg/ spot	-	-	Degradation products	[37]
HPLC	MS-MS	C_{18}	A: (pH 4.60) of 10mM ammonium formate as solvent, B: 0.10% (v/v) HCOOH in ACN	0.25–25.0 ng g^{-1}	1.63 ng g^{-1}	5.43 ng g^{-1}	In human plasma and urine with sildenafil, tadalafil, vardenafil	[53]

Method	Detector	Column/ Stationary phase	Mobile Phase	Linear Range	LOD	LOQ	Matrix	Reference
HPLC	PDA (239 nm)	C_{18}	A: 10 mM NH_4HCO_2 buffer (pH 4.5), B: ACN (A: B, 60:40, v/v)	10–70 mg/ mL	0.36 mg/ mL	1.15 mg/ mL	Stability indicating	[54]
HPLC	MS-MS				-	-		
HPLC	MS	C_{18}	0.1% HCOOH (w/v), and ACN, 29: 71, v/v	50-3200 ng/ml	-	-	Pharmacokinetic study after oral administration and transdermal film application	[55]
HPLC	UV (238 nm)	C_{18}	H_2O, ACN, and CF_3CO_2H in the ratio of (65: 35: 0.1%v/v)	5-100 µg/ ml	-	-	Tablet formulation	[56]
HPLC	MS-MS	C_{18}	Gradient: A, 10mM formate buffer (pH 4.60), B 0.10% (v/v) HCOOH in ACN	-	0.42 ng/g	1.39 ng/g	In human urine and plasma samples	[57]
HPLC	PDA and MS	C_{18}	ACN:buffer [40:60, (v/v); 80:20, (v/v)], pH of buffer (2.5, 6.5)	10–70 µg/ mL	0.360 µg/ mL	1.150 µg/mL	Stability Indicating	[58]
HPLC	MS	C_{18}	A: 0.1% HCOOH, B: ACN 0.1% HCOOH	15.0-6000 ng/mL	-	15.0 ng/ mL	plasma samples	[59]

References

1. Irwin GM. Erectile Dysfunction. *Prim Care*. 2019;46(2):249-255. DOI:10.1016/j.pop.2019.02.006.
2. Mobley DF, Khera M, Baum N. Recent advances in the treatment of erectile dysfunction. *Postgrad Med J*. 2017;93(1105):679-685. DOI:10.1136/postgradmedj-2016-134073.
3. Shamloul R, Ghanem H. Erectile dysfunction. *Lancet*. 2013;381(9861):153-165. DOI:10.1016/S0140-6736(12)60520-0.
4. Mitidieri E, Cirino G, d'Emmanuele di Villa Bianca R, Sorrentino R. Pharmacology and perspectives in erectile dysfunction in man. *Pharmacol Ther*. 2020;208:107493. DOI:10.1016/j.pharmthera.2020.107493.
5. Razdan S, Greer AB, Patel A, Alameddine M, Jue JS, Ramasamy R. Effect of prescription medications on erectile dysfunction. *Postgrad Med J*. 2018;94(1109):171-178. DOI:10.1136/postgradmedj-2017-135233.
6. Ahmed WS, Geethakumari AM, Biswas KH. Phosphodiesterase 5 (PDE5): Structure-function regulation and therapeutic applications of inhibitors. *Biomed Pharmacother*. 2021;134:111128. DOI:10.1016/j.biopha.2020.111128.
7. Carson CC, Lue TF. Phosphodiesterase type 5 inhibitors for erectile dysfunction. *BJU Int*. 2005;96(3):257-280. DOI:10.1111/j.1464-410X.2005.05614.x.
8. Kerr NM, Danesh-Meyer HV. Phosphodiesterase inhibitors and the eye. *Clin Exp Ophthalmol*. 2009;37(5):514-523. DOI:10.1111/j.1442-9071.2009.02070.x.
9. Yéléhé-Okouma M, Pape E, Humbertjean L, et al. Drug adulteration of sexual enhancement supplements: a worldwide insidious public health threat [published online ahead of print, 2021 Jan 22]. *Fundam Clin Pharmacol*. 2021;10.1111/fcp.12653. DOI:10.1111/fcp.12653.
10. da Justa Neves DB, Caldas ED. Dietary supplements: International legal framework and adulteration profiles, and characteristics of products on the Brazilian clandestine market. *Regul Toxicol Pharmacol*. 2015;73(1):93-104. DOI:10.1016/j.yrtph.2015.06.013.
11. Bin Hu, Yingyan Huang, Guo Yin, Gaofei Zhang, Liyang Zhang, Tiejie Wang, Zhong-Ping Yao. Rapid detection of adulterated drugs in herbal dietary supplements by wooden-tip electrospray ionization mass spectrometry. *Anal. Methods*, 2016, **8**, 6840-6846. DOI: 10.1039/C6AY01735E.
12. Avanafil. In: *LiverTox: Clinical and Research Information on Drug-Induced Liver Injury*. Bethesda (MD): National Institute of Diabetes and Digestive and Kidney Diseases; August 2, 2017.
13. Drugs@FDA: FDA-Approved Drugs, https://www.accessdata.fda.gov/scripts/cder/daf/index.cfm?event=overview.process&ApplNo=202276.

ISSN: 2458 - 8806

14. Kedia GT, Uckert S, Assadi-Pour F, Kuczyk MA, Albrecht K. Avanafil for the treatment of erectile dysfunction: initial data and clinical key properties. *Ther Adv Urol*. 2013;5(1):35-41. DOI:10.1177/1756287212466282.

15. Jonathan L Zurawin, Carrie A Stewart, James E Anaissie, Faysal A Yafi, Wayne JG Hellstrom. Avanafil for the treatment of erectile dysfunction, Expert Review of Clinical Pharmacology. Expert Rev Clin Pharmacol. 2016 Sep;9(9):1163-70. DOI: 10.1080/17512433.2016.1212655.

16. Cui YS, Li N, Zong HT, Yan HL, Zhang Y. Avanafil for male erectile dysfunction: a systematic review and meta-analysis. *Asian J Androl*. 2014;16(3):472-477. DOI:10.4103/1008-682X.123670.

17. Preparation method of medical compound avanafil. Assignee: Chongqing Aoshe Biochemical Co ltd. Available online: https://patents.google.com/patent/CN109280050B/en.

18. Michele P. Kelly, Nicholas J. Brandon. Differential function of phosphodiesterase families in the brain: gaining insights through the use of genetically modified animals. Editor(s): Sawa Akira, Progress in Brain Research, Elsevier, Volume 179, 2009, 67-73. DOI: https://doi.org/10.1016/S0079-6123(09)17908-6.

19. Omori K, Kotera J. Overview of PDEs and their regulation. Circ Res. 2007;100(3):309-27. DOI: 10.1161/01.RES.0000256354.95791.f1.

20. Aversa A, Duca Y, Condorelli RA, Calogero AE, La Vignera S. Androgen Deficiency and Phosphodiesterase Type 5 Expression Changes in Aging Male: Therapeutic Implications. Front Endocrinol (Lausanne). 2019;10:225. DOI: 10.3389/fendo.2019.00225.

21. Morales, A.; Gingell, C.; Collins, M.; Wicker, P. A.; Osterloh, I. H., Clinical safety of oral sildenafil citrate (viagra) in the treatment of erectile dysfunction. *Int J Impot Res* **1998,** *10* (2), 69- 73

22. Padma-Nathan, H.; McMurray, J. G.; Pullman, W. E.; Whitaker, J. S.; Saoud, J. B.; Ferguson, K. M.; Rosen, R. C.; Group, I. C. O.-D. D. S., On-demand ic351 (cialis) enhances erectile function in patients with erectile dysfunction. *Int J Impot Res* **2001,** *13* (1), 2-9.

23. Hellstrom, W. J.; Gittelman, M.; Karlin, G.; Segerson, T.; Thibonnier, M.; Taylor, T.; Padma-Nathan, H., Vardenafil for treatment of men with erectile dysfunction: Efficacy and safety in a randomized, double-blind, placebo-controlled trial. *J Androl* **2002,** *23* (6), 763-771.

24. Kedia, G. T.; Uckert, S.; Assadi-Pour, F.; Kuczyk, M. A.; Albrecht, K., Avanafil for the treatment of erectile dysfunction: Initial data and clinical key properties. *Ther Adv Urol* **2013,** *5* (1), 35-41.

25. Hsieh CM, Chen CY, Chern JW, Chan NL. Structure of human phosphodiesterase 5A1 complexed with avanafil reveals molecular basis of isoform selectivity and guidelines for targeting α-helix backbone oxygen by halogen bonding. J Med Chem. 2020;63(15):8485-8494. DOI: 10.1021/acs.jmedchem.0c00853.

26. Allison M, Grant T, Obaidi M, Marenco T, Yee S, Day W. Pharmacokinetics of avanafil; a novel, rapidly absorbed, selective PDE5 inhibitor for the treatment of mild to severe erectile dysfunction. *J Sex Med.* 2011;(8):S466–S467.

27. Katz EG, Tan RB, Rittenberg D, Hellstrom WJ. Avanafil for erectile dysfunction in elderly and younger adults: differential pharmacology and clinical utility. Ther Clin Risk Manag. 2014;10:701-11. DOI: 10.2147/TCRM.S57610.

28. Kalgutkar AS, Driscoll JP. Is there enough evidence to classify cycloalkyl amine substituents as structural alerts? Biochem Pharmacol. 2020;174:113796. DOI: 10.1016/j.bcp.2020.113796.

29. Huang SA, Lie JD. Phosphodiesterase-5 (PDE5) Inhibitors in the Management of Erectile Dysfunction. P T. 2013 Jul;38(7):407-19.

30. Avanafil for erectile dysfunction. Aust Prescr 2019;42:113-4. First published 13 May 2019. DOI: https://doi.org/10.18773/austprescr.2019.043.

31. Nurse's Drug Handbook. 2018, Seventeenth Edition, Jones & Bartlett Learning. Page 102.

32. Analytical Methods Committee Amctb No. Units and quantities for analytical chemistry (background paper). *Anal Methods*. 2020;12(41):5010-5012. DOI:10.1039/d0ay90126a.

33. Hoon Kang, Sang-Ryoul Park, Ju Hwang Kim, In-Yong Park & Haewon Jung (2021): Novel microsampling system for automated spectrophotometry, Instrumentation Science & Technology, DOI: 10.1080/10739149.2021.1950181

34. Raval Kashyap, U.Srinivasa, Kalindi Badodaria. First order derivative and dual wavelength spectrophotometry methods development and validation for simultaneous estimation of avanafil and dapoxentine hydrochloride in bulk and dosage form. International Journal of Pharmacy & Technology. 2014, Vol. 6, Issue No.1, 6418-6438.

35. Pratik M. Tailor, Vrutika V. Patel, Ashish D. Mishra, Shailesh A. Shah, Dinesh R. Shah. Spectrofluorimetric Method for the Estimation of Avanafil in Bulk and their Tablet Dosage Form. RRJoDFDP (2016) 17-21.

36. Mital N Patel, Charmy S Kothari, Multivariate Approaches for Simultaneous Determination of Avanafil and Dapoxetine by UV Chemometrics and HPLC-QbD in Binary Mixtures and Pharmaceutical Product, *Journal of AOAC INTERNATIONAL*, Volume 99, Issue 3, 1 May 2016, Pages 649–663, https://doi.org/10.5740/jaoacint.15-0259

37. Darwish, M. K., Soliman, M. M., & Abdel-Razeq, S. A. (2019). Validated Stability Indicating HPTLC and UV-

Spectrophotometric Techniques for the Determination of Avanafil. Asian Journal of Chemical Sciences, 6(4), 1-16. https://doi.org/10.9734/ajocs/2019/v6i419005.

38. T.A. Mohamed and S.A. Atty, Native and synchronous fluorescence spectroscopy for determination of avanafil in presence of its co-formulated drug (dapoxetine hydrochloride): Application to pharmaceutical product, biological fluid and content uniformity, *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy* (2019), https://doi.org/10.1016/j.saa.2019.117898.
39. Abdel-Raoof, A.M., Said, R.A.M., Emara, M.S., El-Desouky, E.A., Abdelzaher, A.M., Hasan, M.A., Osman, A.E. D-optimal design as a useful tool response surface methodology for the optimization of signals from synchronous fluorescence prior to simultaneous determination of avanafil and tadalafil. Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy 235 (2020) 118313. DOI: https://doi.org/10.1016/j.saa.2020.118313.
40. Khalid A. M. Attia, Ahmad A. Mohamad, Mohamed S. Emara, Ahmed M. Abdel-Raoof, Mohamed A. Hasan, Ahmed W. Madkour, Ebrahim A. El-Desouky. Second derivative synchronous fluorescence determination of avanafil in the presence of its acid-induced degradation product aided by powerful Lean Six Sigma tools augmented with D-optimal design. RSC Adv., 2021, 11, 3834. DOI: https://doi.org/10.1039/D0RA08216C.
41. Kjell O. Eriksson. Reversed Phase Chromatography, Editor(s): Günter Jagschies, Eva Lindskog, Karol Łącki, Parrish Galliher, Biopharmaceutical Processing, Elsevier, 2018, 433-439. DOI: https://doi.org/10.1016/B978-0-08-100623-8.00022-0.
42. John W. Dolan, Lloyd R. Snyder. Method development in liquid chromatography, Editor(s): Salvatore Fanali, Paul R. Haddad, Colin F. Poole, Marja-Liisa Riekkola, Liquid Chromatography (Second Edition), Elsevier, 2017, Pages 375-388. DOI: https://doi.org/10.1016/B978-0-12-805393-5.00014-2.
43. Chaudhery MH, Deepak R, Gaurav P, Maithri T. Handbook of Analytical Techniques for Forensic Samples. Current and Emerging Developments. 2021, 169-187. DOI: https://doi.org/10.1016/B978-0-12-822300-0.00010-0.
44. Jung, J., Choi, S., Cho, S. H., Ghim, J. L., Hwang, A., Kim, U., Kim, B. S., Koguchi, A., Miyoshi, S., Okabe, H., Bae, K. S., & Lim, H. S. (2010). Tolerability and pharmacokinetics of avanafil, a phosphodiesterase type 5 inhibitor: a single- and multiple-dose, double-blind, randomized, placebo-controlled, dose-escalation study in healthy Korean male volunteers. *Clinical therapeutics*, *32*(6), 1178–1187. DOI: https://doi.org/10.1016/j.clinthera.2010.06.011.
45. M. Hegazy, A. Kessiba, M. Abdelkawy, A. E. El-Gindy. RP-HPLC with Time Programmed Fluorescence Detection for Quantitation of Avanafil and Dapoxetine Hydrochloride: Application to Pharmaceutical Dosage Form and Biological Fluid, Journal of Liquid Chromatography & Related Technologies, 2015;38:18, 1660-1665, DOI: 10.1080/10826076.2015.1087860.
46. Nitin Kumar, D. Sangeetha, L. Kalyanraman, K. Sainath. Stability-indicating HPLC method for simultaneous determination of degradation products and process-related impurities of avanafil in avanafil tablets. Acta Chromatographica 30(2018)3, 158–163. DOI: 10.1556/1326.2017.00116
47. Usama A. Fahmy, Bader M. Aljaeid. Stability indicating HPLC method for analysis of Avanafil using diode array detector. International Journal of Advances in Pharmacy, Biology and Chemistry, 5(1), 2016, 59-64.
48. Pavani Peddi, T. Raja Rajeswari, Ramana Reddy Ganji. Development and validation of stability indicating HPLC method for estimation of related substances in avanafil. Indo American Journal of Pharmaceutical Research.2017:7(04), 8318-8329.
49. Wu Yuanyang, Wang Ren, Zhou Minghao, Cheng Qiaoyuan. Detection of Avanafil and Flibanserin in Health Food by HPLC-MS/MS. Herald of Medicine 2017, 36(7): 783-785. DOI: 10.3870/j.issn.1004-0781.2017.07.015.
50. Wang, X. B., Zheng, J., Li, J. J., Yu, H. Y., Li, Q. Y., Xu, L. H., Liu, M. J., Xian, R. Q., Sun, Y. E., & Liu, B. J. (2018). Simultaneous analysis of 23 illegal adulterated aphrodisiac chemical ingredients in health foods and Chinese traditional patent medicines by ultrahigh performance liquid chromatography coupled with quadrupole time-of-flight mass spectrometry. *Journal of food and drug analysis*, *26*(3), 1138–1153. https://doi.org/10.1016/j.jfda.2018.02.003
51. Can N. Ö. (2018). Development of Validated and Stability-Indicating LC-DAD and LC-MS/MS Methods for Determination of Avanafil in Pharmaceutical Preparations and Identification of a Novel Degradation Product by LCMS-IT-TOF. *Molecules (Basel, Switzerland)*, *23*(7), 1771. https://doi.org/10.3390/molecules23071771
52. Öztürk Er, E., Özbek, B., & Bakırdere, S. (2019). Accurate and sensitive determination of sildenafil, tadalafil, vardenafil, and avanafil in illicit erectile dysfunction medications and human urine by LC with quadrupole-TOF-MS/MS and their behaviors in simulated gastric conditions. *Journal of separation science*, *42*(2), 475–483. https://doi.org/10.1002/jssc.201800734
53. Elif Ozturk Er, Erhan Akkaya, Belma Ozbek, Sezgin Bakırdere. Development of an analytical method based on citric acid coated magnetite nanoparticles assisted dispersive magnetic solid-phase extraction for the enrichment and extraction of sildenafil, tadalafil, vardenafil and avanafil in human plasma and urine prior to determination by LC-MS/MS. Microchemical Journal 147 (2019) 269–276. DOI: https://doi.org/10.1016/j.microc.2019.03.043.

ISSN: 2458 - 8806

54. Mital Patel, Charmy Kothari, Vivek Vyas. An innovative impurity profiling of Avanafil using LC and LC-MS/MS with in-silico toxicity prediction. Arabian Journal of Chemistry (2020) 13, 6493–6509. DOI: https://doi.org/10.1016/j.arabjc.2020.06.007.

55. Ahmed K. Kammoun, Alaa Khedr, Osama A. A. Ahmed. LC-MS/MS determination of avanafil and its metabolites in rat plasma and brain: pharmacokinetic study after oral administration and transdermal film application. RSC Adv., 2020, 10, 9407. DOI: https://doi.org/10.1039/D0RA00569J.

56. M. V. Jadhav, A. Akash, V. Munipalli, R.M. Singh, S. Nayak, B. Vaidhun. Development and Validation of Reverse Phase High Performance Liquid Chromatography Method for Quantitative Estimation of Avanafil in Tablet Dosage Form. Journal of Pharma Innovation. 1(1), 2020, 29-37.

57. Elif Ozturk Er, Erhan Akkaya, Belma Ozbek, Sezgin Bakırdere. A powerful combination of quadruple isotope dilution strategy with dispersive magnetic solid phase extraction for the accurate and precise multi-analyte determination of tadalafil, sildenafil, avanafil and vardenafil in human plasma and urine samples using LC-ESI-Tandem MS. Microchemical Journal 152 (2020) 104302 DOI: https://doi.org/10.1016/j.microc.2019.104302.

58. Patel, M., Kothari, C. Comprehensive stability-indicating method development of Avanafil Phosphodiesterase type 5 inhibitor using advanced Quality-by-Design approach. *J Anal Sci Technol* **11,** 29 (2020). https://doi.org/10.1186/s40543-020-00228-4.

59. Ren, X., Yu, H., Qi, X., Chen, Q., Yang, J., Fang, Y., Lei, Y., Zhang, D., Zuo, Q., & Liu, D. (2021). A Bioequivalence Study of Avanafil in Healthy Chinese Male Subjects Under Fasting and Fed Conditions: Results of a Randomized, Open-Label, Single-Dose, 2-Sequence, 2-Period Crossover Study. *Clinical pharmacology in drug development*, 10.1002/cpdd.998. https://doi.org/10.1002/cpdd.998.

60. Fernández-Maestre, Roberto. (2012). Ion mobility spectrometry: History, characteristics and applications. *Revista U.D.C.A Actualidad & Divulgación Científica*, *15*(2), 467-479.

61. Mans, D. J., Callahan, R. J., Dunn, J. D., & Gryniewicz-Ruzicka, C. M. (2013). Rapid-screening detection of acetildenafils, sildenafils and avanafil by ion mobility spectrometry. *Journal of pharmaceutical and biomedical analysis*, *75*, 153–157. https://doi.org/10.1016/j.jpba.2012.11.031.

62. Kyle JA, Brown DA, Hill JK. Avanafil for erectile dysfunction. Ann Pharmacother. 2013 Oct;47(10):1312-20. doi: 10.1177/1060028013501989.

63. Shalender Bhasin, Rosemary Basson. Sexual Dysfunction in Men and Women, Editor(s): Shlomo Melmed, Kenneth S. Polonsky, P. Reed Larsen, Henry M. Kronenberg, Williams Textbook of Endocrinology (Thirteenth Edition), Elsevier, 2016, 785-830. DOI: https://doi.org/10.1016/B978-0-323-29738-7.00020-4.

64. Ji Hye Jeong, Ji Hyun Lee, Hyung Joo Kim, Hyoung Joon Park, In Sun Hwang, Kyoung Moon Han, Chang-Yong Yoon, Sooyeul Cho & Woo Seong Kim (2014): LC-ESI-MS/MS analysis of phosphodiesterase-5 inhibitors and their analogues in foods and dietary supplements in Korea, Food Additives & Contaminants: Part B: Surveillance, DOI: 10.1080/19393210.2014.968220.

65. Wang R, Zhou M, Cheng Q (2017) Detection of Avanafil and Flibanserin in Health Food by HPLC-MS/MS. Herald of Medicine 36(7): 783-785.

66. Serban C. Moldoveanu, Victor David. The Practice of HPLC, Editor(s): Serban C. Moldoveanu, Victor David, Selection of the HPLC Method in Chemical Analysis, Elsevier, 2017, 463-470. DOI: https://doi.org/10.1016/B978-0-12-803684-6.00015-9.

67. Aubry AF., Tattersall P., Ruan J. (2009) Development of Stability Indicating Methods. In: Huynh-Ba K. (eds) Handbook of Stability Testing in Pharmaceutical Development. Springer, New York, NY. DOI: https://doi.org/10.1007/978-0-387-85627-8_7.

68. Paweł Mateusz Nowak, Renata Wietecha-Posłuszny, Janusz Pawliszyn. White analytical chemistry: an approach to reconcile the principles of green analytical chemistry and functionality. Trends in Analytical Chemistry. Volume 138, May 2021, 116223. DOI: https://doi.org/10.1016/j.trac.2021.116223.

69. Aldawsari, H. M., Fahmy, U. A., Abd-Allah, F., & Ahmed, O. (2020). Formulation and Optimization of Avanafil Biodegradable Polymeric Nanoparticles: A Single-Dose Clinical Pharmacokinetic Evaluation. *Pharmaceutics*, *12*(6), 596. DOI: https://doi.org/10.3390/pharmaceutics12060596.

70. National Center for Biotechnology Information (2021). PubChem Compound Summary for CID 9869929, Avanafil. Retrieved September 22, 2021 from https://pubchem.ncbi.nlm.nih.gov/compound/Avanafil.

71. Amit Kumar Nayak, Prachi Prava Panigrahi. Solubility Enhancement of Etoricoxib by Cosolvency Approach. ISRN Physical Chemistry, Volume 2012, Article ID 820653, DOI: https://doi.org/10.5402/2012/820653.

72. Pitt JJ. Principles and applications of liquid chromatography-mass spectrometry in clinical biochemistry. *Clin Biochem Rev*. 2009;30(1):19-34.

73. Shrivastava A, Mittal A. A Mini Review on Characteristics and Analytical Methods of Otilonium Bromide. Crit Rev Anal Chem. 2021 May 26:1- 9. DOI: 10.1080/10408347.2021.1913983.

74. Shrivastava Alankar, Mittal Ashu, Khabiya Rakhi, Choudhary GP and Darwhekar N. Gajanan, Brexpiprazole: Characteristics, Biological Activities, Synthesis and Methods for Determination in Different Matrices, Current Pharmaceutical Analysis 2022; 18. DOI: https://dx.doi.org/10.2174/1573412918666220406123600.

75. Hernandez, I., Gul, Z., Gellad, W. F., & Davies, B. J. (2021). Marked Increase in Sales of Erectile Dysfunction Medication During COVID-19. Journal of general internal medicine, 36(9), 2912–2914. DOI: https://doi.org/10.1007/s11606-021-06968-2.

ISSN: 2458 - 8806