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PHOSPHODIESTERASE 5 INHIBITORS: A REVIEW OF ANALYTICAL METHODS

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ABSTRACT

A **phosphodiesterase type 5 inhibitor** (**PDE5 inhibitor**) is a vasodilating drug which works by blocking the degradative action of cGMP-specific phosphodiesterase type 5 (PDE5) on cyclic GMP in the smooth muscle cells lining the blood vessels supplying various tissues. These drugs dilate the corpora cavernosa of the penis, facilitating erection with sexual stimulation, and are used in the treatment of erectile dysfunction (ED). Sildenafil was the first effective oral treatment available for ED. Because PDE5 is also present in the smooth muscle of the walls of the arterioles within the lungs, two PDE5 inhibitors, sildenafil and tadalafil, are FDA-approved for the treatment of pulmonary hypertension. As of 2019, the wider cardiovascular benefits of PDE5 inhibitors are being appreciated

Key Words: A phosphodiesterase type 5 inhibitor (PDE5 inhibitor, Sildenafil, tadalafil, Avanafil, mirodenafil, udenafil and lodenafil, Zaprinast

INTRODUCTION:

Erectile dysfunction (impotence) is the inability to get and keep an erection firm enough for sex.Having erection trouble from time to time isn't necessarily a cause for concern. If erectile dysfunction is an ongoing issue, however, it can cause stress, affect your self-confidence and contribute to relationship problems. Problems getting or keeping an erection can also be a sign of an underlying health condition that needs treatment and a risk factor for heart disease. **Cyclic guanosine monophosphate-specific phosphodiesterase type 5** is an enzyme (EC 3.1.4.17) from the phosphodiesterase class. It is found in various tissues, most prominently the corpus cavernosum and the retina. It has also been recently discovered to play a vital role in the cardiovascular system.The phosphodiesterase (PDE) isozymes, found in several tissues including the rod and cone photoreceptor cells of the retina, belong to a large family of cyclic nucleotide PDEs that catalyze cAMP and cGMP hydrolysis. (1,2)

The interest in PDEs as molecular targets of drug action has grown with the development of isozyme-selective PDE inhibitors that offer potent inhibition of selected isozymes without the side-effects attributed to nonselective inhibitors such as theophylline. Sildenafil, vardenafil, tadalafil, and avanafil are PDE5 inhibitors that are significantly more potent and selective than zaprinast and other early PDE5 inhibitors.

PDE5 is an enzyme that accepts cGMP and breaks it down. Sildenafil, vardenafil and tadalafil are inhibitors of this enzyme, which bind to the catalytic site of PDE5. Both inhibitors bind with high affinity and specificity, and cGMP-binding to the allosteric sites stimulates binding of PDE5 inhibitors at the catalytic site. The kinetics of inhibitor binding and inhibition of catalysis imply the existence of two PDE5 conformers, and results of native gel electrophoresis reveal that PDE5 exists in two apparently distinct conformations, i.e., an extended conformer and a more compact conformer.

PDE5 activity is modulated by a rapidly reversible redox switch. Chemical reduction of PDE5 relieves autoinhibition of enzyme functions; allosteric cGMP-binding activity is increased 10-fold, and catalytic activity is increased ~3-fold. The redox effect on allosteric cGMP-binding occurs in the isolated regulatory domain. A change in the state of reduction of PDE5 or the isolated regulatory domain is associated with an apparent conformational change similar to that caused by phosphorylation.

PDE5 is expressed in human colonic cells and in intestinal tissue and its activity is regulated by intracellular cGMP levels in these cells that increase on GCC activation. This presumably occurs through binding of cGMP to the GAF domains in the N-terminus of PDE5, resulting in allosteric activation of the enzyme.

The mechanism of action of E4021 on both the nonactivated and activated forms of rod PDE6 because both states are relevant to understanding how PDE5-selective inhibitors may alter signal transduction pathways in photoreceptor cells. PDE5-selective inhibitors may show good discrimination of PDE5 from most other PDE isoforms.

In addition to human corpus cavernosum smooth muscle, PDE5 is also found in lower concentrations in other tissues including platelets, vascular and visceral smooth muscle, and skeletal muscle. The inhibition of PDE5 in these tissues by sildenafil may be the basis for the enhanced platelet antiaggregatory activity of nitric oxide observed in vitro, an inhibition of platelet thrombus formation in vivo and peripheral arterial-venous dilatation in vivo.

Immunohistology has shown that PDE5 localizes in heart cells at the sarcomere z-disk, but can also be found in diffuse amounts in the cytosol. Increased expression of PDE5 has also been measured in hypertrophic disease and has been linked to oxidative stress, and PDE5 inhibition has shown beneficial effects in the failing heart. In an experiment, PDE5 overexpression was found to contribute to worsened pathological remodeling after mouse cardiomyocytes experienced myocardial infarction. The role of PDE5 in heart failure and cardiac treatment involving PDE5 inhibitors have been major areas of focus for both lab and clinical studies. (1,2).

PDE5 INHIBITOR

A phosphodiesterase type 5 inhibitor (PDE5 inhibitor) is a vasodilating drug which works by blocking the degradative action of cGMP-specific phosphodiesterase type 5 (PDE5) on cyclic GMP in the smooth muscle cells lining the blood vessels supplying various tissues. These drugs dilate the corpora cavernosa of the penis, facilitating erection with sexual stimulation, and are used in the treatment of erectile dysfunction (ED). Sildenafil was the first effective oral treatment available for ED. Because PDE5 is also present in the smooth muscle of the walls of the arterioles within the lungs, two PDE5 inhibitors, sildenafil and tadalafil, are FDA-approved for the treatment of pulmonary hypertension. As of 2019, the wider cardiovascular benefits of PDE5 inhibitors are being appreciated.

CONTRAINDICATIONS

PDE5 inhibitors are contraindicated within 24 hours (or 48 hours with tadalafil) of taking alpha-blockers, soluble guanylate cyclase stimulators, or nitrate medications such as isosorbide mononitrate or isosorbide dinitrate. Concurrent use of these medications can lead to life-threatening low blood pressure. PDE5 inhibitors are also contraindicated in patients with previous nonarteritic anterior ischaemic optic neuropathy and hereditary eye diseases.
Despite initial concerns of adverse cardiovascular events in patients prescribed PDE5 inhibitors, several long-term studies have established the safety of the drugs in both healthy patients and patients with cardiovascular risk factors.

MEDICAL USES

Phosphodiesterase-5 (PDE5) inhibitors such as sildenafil (Viagra), tadalafil (Cialis), and vardenafil (Levitra) are clinically indicated for the treatment of erectile dysfunction. Sildenafil and tadalafil are also indicated for the treatment of some subtypes of pulmonary hypertension, while tadalafil is also licensed for the treatment of benign prostatic hyperplasia. PDE5 inhibitors have been used as a second line therapy in severe cases of Raynaud phenomenon when it is related to systemic sclerosis per The European Society for Vascular Medicine guidelines. Sildenafil, the prototypical PDE5 inhibitor, was originally discovered during the search of a novel treatment for angina. Studies in 2002 explored its potential for increasing neurogenesis after stroke, but clinical evidence for benefit in cerebrovascular diseases is currently lacking. (1,2)

MECHANISM OF ACTION

Part of the physiological process of vasodilatation involves the release of nitric oxide (NO) by vascular endothelial cells which then diffuses to nearby vascular smooth muscle cells. There, NO activates soluble guanylate cyclase which converts guanosine triphosphate (GTP) to cyclic guanosine monophosphate (cGMP), the main effector of the system. For example, in the penis, NO release at high levels from endothelial cells and penile nerves during sexual stimulation leads to relaxation of the smooth vasculature of the corpus cavernosum, causing vasocongestion and a sustained erection.[1]
PDE5 inhibitors prolong the action of cGMP by inhibiting its degradation by the enzyme PDE5, which is found throughout the body. In the penis, PDE5 inhibitors potentiate the effects of cGMP to prolong erections and increase sexual satisfaction.[12] However, PDE5 inhibitors do not cause erections without sexual stimulation.

As well as their haemodynamic effects, PDE5 inhibitors have also been shown to have anti-inflammatory, antioxidant, antiproliferative, and metabolic properties in several experiments. However, larger and longer-term studies are needed to establish their effectiveness and safety compared to other medications in other diseases.

OVERVIEW OF ANALYTICAL METHODS FOR DETERMINATION OF PHOSPHODIESTERASE 5 INHIBITORS IN PHARMACEUTICAL FORMULATION.

Analytical Methods for PDE-5 Inhibitors Detection

Several analytical methods are suitable to estimate the concentration of PDE-5 inhibitors: spectrophotometric methods ,capillary electrophoretic methods ,atomic emission and atomic absorption spectrometry methods and chromatographic techniques (GC-LC-HPLC-UHPLC) coupled with different detectors, including mass spectrometers (MS) .Only a few reports are available in the literature for the GC-MS use to analyse PDE-5 inhibitors, maybe due to the thermal instability of these compounds and their analogues and the difficulties in derivatising them with standard silylation reagents .Liquid chromatography techniques, instead, seem to be more suitable for the analysis of PDE-5 inhibitors.

TABLE 1: REPRESENTATIVE OF ANALYTICAL METHODS FOR DETERMINATION OF PHOSPHODIESTERASE 5 INHIBITORS

COMPOUNDS	METHODS	ABSORPSTION MAXIMUM	SOLVENT	LIMIT OF DETECTION	REFERENCES NO
Ambrisentan and Tadalafil	HPLC	260 nm,	A mobile phase consisting of buffer (PH 3): methanol (50:50)	Ambrisentan-0.0096 µg/ml, Tadalafil-0.117 µg/ml,	4
Avanafil	The HPLC stabililty study with Quality-by-Design (QbD) approach	239 nm.	The mobile phase [10 mMammonium acetate, pH 4.5 adjusted by acetic acid:CAN (60:40 v/v) at 0.9 ml/min flow rate	0.360 µg/mL	5
Sildenafil	worked on "Investigation of the presence of Sildenafil in Herbal Dietary Supplements by Validated HPLC Method".	293 nm.	The mobile phase used was 10mM phosphate buffer containing 0.1% triethylamine (pH 3.5) and acetonitrile (65:35 v/v) as mobile phase was	LOD (1.9ng /mL)	6
Sildenafil Citrate and Depoxetine Hydrochlorife	"Development of reverse phase high performance liquid chromatography method for simultaneous estimation of Sildenafil Citrate and Depoxetine Hydrochlorife in Pharmaceutical Formulation".	292 nm and 231 nm,	mixture of Buffer pH 4.0 and CAN in the ratio of 40:60 as the mobile phase	Sildenafil Citrate 0.5282 µg/mL, Depoxetine Hydrochlorife 0.0904 µg/mL	7

Sildenafil Citrate and Depoxetine Hydrochlorife	Uv-Analytical Method Development And Validation For Simultaneous Estimation Of Dapoxetine Hydrochloride And Sildenafil Citrate In Tablet Dosage Form	292 nm and 231 nm,	methanol	Sildenafil Citrate 0.5282 µg/mL, Depoxetine Hydrochlorife 0.0904 µg/mL	8
Sildenafil Citrate	An improved RP-HPLC method for the Quantitative determination and validation of Sildenafil Citrate in bulk and pharmaceutical formulation	230 nm	mobile phase containing 10 mM Phosphate buffer: acetonitrile (50:50 v/v) adjusted to pH 3.0utilizing orthophosphoric acid	0.4221 µg/ml	9
Sildenafil Tartrate	HPLC determination of Sildenafil Tartrate and its related substances along with some Supportive Studies using MS, XRD and NMR".	290 nm	Methanol was used as diluent and mobile phase used was 0.1% formic acid and methanol (30:70 ratio).		10
Sildenafil Citrate	"Analytical Method Development and Validation of Sildenafil Citrate by RP-HPLC". For the analysis of Sildenafil Citrate in pharmaceutical formulations, a simple,	230 nm	Acetonitrile/Phosphate buffer mobile phase (35:65, v/v).	0.03µg/ml	11

	accurate, reverse phase - high performance liquid chromatographic (RP-HPLC)				
Sildenafil Citrate	Validation of Simple and Rapid UV-Spectrophotometric Method with Stress Degradation Study for Sildenafil Citrate	228 nm	Methanol	---	12
Tadalafil and Sildenafil	Method development and validation of stability indicating methods for assay of Tadalafil and Sildenafil citrate by HPLC	220nm	phosphate buffer (10mM,pH 3.0) acetonitrile gradient, methanol	-	13
Sildenafil	Spectrophotmetric Estimation of Sildenafil Citrate in tablets	715 nm,700 nm	potassium ferricyanide, potassium dichromate	-	14
sildenafil citrate	Development and validation of RP-HPLC method for sildenafil citrate in rat plasma-application to pharmacokinetic studies	230 nm.	methanol:water (85:15 v/v) as mobile phase	0.1–6 µg/ml	15

vardenafil	"Method development and validation of vardenafil in bulk drug form using RP-HPLC".	300 nm	methanol: water (80:20)	0.01 and	16
Tadalafil	Validation and method development of Tadalafil in bulk and tablet dosage form by RP-HPLC	285 nm	buffer (potassium dihydrogen orthophosphate) and acetonitrile in the ration of 50:50 V/V,	----	17
Tadalafil	Determination of tadalafil in pure powder and tablet dosage form by high-performance liquid chromatography	225 nm	buffer-acetonitrile (70 + 30, v/v), adjusted to pH 7.00 +/- 0.05 with triethylamine as the mobile phase	50.7-152.10 microg/mL	18
Tadalafil	worked on "Development and Validation of Tadalafil Determination in Human Plasma by HPLC-MS Method".		acetonitrile protein precipitation,	-	19
Tadalafil	- worked on "RP-HPLC-PDA method development and validation for the analysis of Tadalafil in bulk, pharmaceutical dosage forms and in-vitro dissolution samples	280 nm	Ammonium acetate (10 mM): methanol (35:65v/v)	---	20

sildenafil	worked on- "RP-HPLC method development for estimation of sildenafil citrate in tablets and seminal fluid	230 nm	With OPA and ACN (60:40) and a flow rate of 1 ml/min, the mobile phase TEA (0.2 percent) was adjusted to a pH of 3 in the process	0.03µg/mL	21
Avanafil	worked on "Development and validation of reverse phase high performance liquid chromatography method for quantitative estimation of Avanafil in tablet dosage form	238 nm	mobile phase composition of water, acetonitrile and trifluroacetic acid in the ratio of (65:35:0.1 % v/v) was used.		22
Avanafil".	worked on"Validated stability indicating HPTLC and UV-Spectrophotometric techniques for the determination of Avanafil". UV-Visible spectrophotometry	267 and 292 nm. UV detection at 230 nm	with chloroform: toluene: methanol: conc. ammonia (6:5:3:0.1, by volume)	------	23
avanafil	worked on "Stability-indicating HPLC method for simultaneous determination of degradation products and process-related impurities of avanafil in avanafil tablets".	245 nm	Mobile phase A, 0.1% trifluro acetic acid and triethylamine in water, and mobile phase B, water and acetonitrile in the ratio of 20:80 (v/v) were used at a flow rate of 1.2ml/min in gradient elution mode.	0.0352µg/mL	24

Avanafil and Dapoxetine Hydrochloride	Validated Capillary Zone Electrophoretic Determination of Avanafil and Dapoxetine Hydrochloride in their Pure form and Pharmaceutical Preparation	210 nm for DAP and 248 nm for AVA and VAR	100 mM acetate buffer at pH 3.6.	The limit of detection (LOD) was 0.523 and 0.531 for AVA and DAP	25
lodenafil	lodenafil carbonate using liquid chromatography MS/MS	--	A=H2O-ammonium acetate 50 mM; B=CH3CN-ammonium acetate 50 mM)/ 1.5 mL/min/gradient	--	26
Mirodenafil	High-sensitive LC-MS/MS method for the simultaneous determination of mirodenafil and its major metabolite, SK-3541, in human plasma: application to microdose clinical trials of mirodenafil	-	5 mM ammonium formate and ACN (23:77, v/v) at a flow rate of 0.35 mL/min.	-	27
udenafil	Simultaneous determination of udenafil and its active metabolite, DA-8164, in human plasma and urine using ultra-performance liquid chromatography-tandem mass spectrometry: application to a pharmacokinetic study	----	consisting of acetonitrile and containing 0.1% formic acid (75:25, v/v)	1 ng/mL	28

CONCLUSION:

A large number of techniques are available for the estimation of **Phosphodiesterase 5 Inhibitors** in pharmaceutical formulations. The survey of analytical method data revealed the HPLC, UV, Colorimetry, IR, HPTLC methods which could be used for the estimation of drug alone or in combination with other drugs in various formulations. Thus this article examines published analytical techniques that are reported so far for determination of **Phosphodiesterase 5 Inhibitors** in bulk and pharmaceutical formulations.

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CONFLICT OF INTEREST:

Declared non

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