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A review of analytical methods for the determination of four new phosphodiesterase type 5 inhibitors in biological samples and pharmaceutical preparations

Cristiane Franco Codevilla, Tamara dos Santos Castilhos, Ana Maria Bergold*

Faculty of Pharmacy, Federal University of Rio Grande do Sul, Porto Alegre, Rio Grande do Sul, Brazil

The introduction of oral phosphodiesterase type 5 inhibitor therapy in 1998 revolutionized the treatment of erectile dysfunction. Erectile dysfunction is the most common sexual problem in men. It often has a profound effect on intimate relationships and quality of life. The analysis of pharmaceuticals is an important part of the drug development process as well as for routine analysis and quality control of commercial formulations. Whereas the determination of sildenafil citrate, vardenafil and tadalafil are well documented by a variety of methods, there are few publications about the determination of udenafil, lodenafil carbonate, mirodenafil and avanafil. The paper presents a brief review of the action mechanism, adverse effects, pharmacokinetics and the most recent analytical methods that can determine drug concentration in biological matrices and pharmaceutical formulations of these four drugs.

Uniterms: Phosphodiesterase type 5 inhibitors/determination/pharmaceutical preparations. Phosphodiesterase type 5 inhibitors/determination/biological samples. Analytical methods. Erectile dysfunction.

A introdução da terapia oral com inibidores da fosfodiesterase tipo 5, em 1998, revolucionou o tratamento da disfunção erétil. A disfunção erétil é o problema sexual mais comum em homens. Muitas vezes tem um efeito profundo nas relações íntimas e na qualidade de vida. A análise de produtos farmacêuticos é uma parte importante do processo de desenvolvimento de fármacos, bem como para a análise de rotina e controle de qualidade das formulações comerciais. Enquanto a determinação do citrato de sildenafila, vardenafila e tadalafila está bem documentada por uma variedade de métodos, existem poucas publicações sobre a determinação de udenafila, carbonato de lodenafila, mirodenafila e avanafila. O artigo apresenta uma breve revisão do mecanismo de ação, efeitos adversos, farmacocinética e os mais recentes métodos analíticos, que podem determinar a concentração do fármaco em matrizes biológicas e formulações farmacêuticas destes quatro fármacos.

Unitermos: Inibidores da fosfodiesterase tipo 5/determinação/preparações farmacêuticas. Inibidores da fosfodiesterase tipo 5/determinação/matrizes biológicas Métodos analíticos. Disfunção erétil.

INTRODUCTION

The National Institutes of Health (NIH) Consensus Development Conference on Impotence defined erectile dysfunction (ED) as the inability to achieve or maintain an erection sufficient for satisfactory sexual performance (NIH, 1993). The Massachusetts Male Aging Study (MMAS), the first large community-based observational survey of men aged 40 to 70 years, has demonstrated a combined prevalence of minimal, moderate and complete ED in 52% of men. The annual incidence rate (cases per 1,000 man-years) increased with each decade of age and this rate was different for men 40-49 (12.4), 50-59 (29.8) and 60-69 (46.4) years old (Johannes *et al.*, 2000). The projection for 2025 shows that approximately 322 million men will have ED, with the largest projected increases in the developing world, i.e., Africa, Asia, and South America (Ayatac *et al.*, 1999). In Brazil, the overall incidence rate of ED was 65.6 cases per 1000 persons/year. The estimate for Brazilian men 40 to 69 years old was approximately

***Correspondence:** A. M. Bergold. Faculdade de Farmácia, Universidade Federal do Rio Grande do Sul. Avenida Ipiranga, 2752/704, 90610-000 - Porto Alegre-RS, Brasil. E-mail: ana.bergold@ufrgs.br

1,025,600 new cases per year. The Brazilian estimate was greater than the MMAS study, maybe because the MMAS population was healthier than Brazilian study sample, like fewer smokers, fewer men with heart diseases and hypertension (Moreira *et al.*, 2003).

Erectile dysfunction is often assumed to be a natural concomitant of the aging process, to be tolerated along with other conditions associated with aging. This assumption may not be entirely correct (Nih, 1993). Other causes of ED were well described over the years, including neurogenic, endocrinological and arteriogenic sources (Berookhim, Bar-Chama, 2011).

There are several disorders associated with ED, including hypertension, hyperlipidemia, testosterone deficiency/hypogonadism, diabetes, cardiovascular disease, obesity, lower urinary tract disorders associated with lower urinary tract symptoms (LUTS), depression, alcohol abuse, smoking, chronic obstructive pulmonary disease (Levine, 2000; Foresta *et al.*, 2008; Singh *et al.*, 2009; Aversa *et al.*, 2010; Berookhim, Bar-Chama 2011; Vignera *et al.*, 2012).

The most exciting change in the treatment options available for patients with ED are the oral therapies (Levine, 2000). In 1998, a new class of drugs, the phosphodiesterase type 5 inhibitors (PDE5i) was introduced. PDE5i represent the first-line oral therapy for ED and the success of PDE5i therapy has dramatically changed the management of erectile dysfunction over the past decade (Gratz *et al.*, 2004; Eardley, 2006; Williams, Melman, 2012).

Sildenafil citrate (Viagra®) was the first drug approved for the treatment of ED in 1998. The United States Food and Drug Administration (FDA) approved tadalafil (Cialis®) and vardenafil hydrochloride (Levitra®) in 2003 (Zou *et al.*, 2006; De Orsi *et al.*, 2009). Lodenafil carbonate (Helleva®) is PDE5i developed in Brazil. It is a dimer that acts as a prodrug delivering lodenafil *in vivo* (Toque *et al.*, 2008; Glina *et al*., 2009).

Recently, other PDE5i were developed. Udenafil (Zydena®) is also a potent and selective PDE5i developed by Dong-A Pharmaceutical Company in Korea (Kim *et al.*, 2008; Han *et al.*, 2010). It has not yet been approved by FDA or the European Medicines Agency (EMEA) and was only approved by the Korean Food and Drug Administration (KFDA), being currently used in Korea and Russia (Alwaal *et al.*, 2011; Cho *et al.*, 2012). Mirodenafil (Mvix®), recently marketed in South Korea (Choi *et al.*, 2009), is reported to have an excellent profile of efficacy for erectile dysfunction (Lee *et al.*, 2009; Kim *et al.*, 2010). One of the recently developed PDE5i, avanafil is a promising medication for ED due to its favorable pharmacokinetics, safety, and efficacy (Jung *et al.*, 2010; Alwaal *et al.*, 2011). FDA approved avanafil (Stendra®) in April 27 (Traynor, 2012).

The first and most extensively investigated agent is sildenafil citrate (McNamara; Donatucci, 2011). There are several studies in the literature reporting the determination of sildenafil citrate in pharmaceuticals, plasma samples, herbal drugs or dietary supplements using liquid chromatography (LC) methods (Eerkes *et al.*, 2002; Sheu *et al.*, 2003; Wang *et al.*, 2005; Reepmeyer, Woodruff, 2007; Reddy, Reddy, 2008; Ortiz *et al.*, 2010; Bartošová *et al.*, 2011; Hasegawa *et al.*, 2012), gas chromatography (GC) (Berzas *et al.*, 2002; Kim *et al.*, 2003a; Man *et al.*, 2009; Strano-Rossi *et al.*, 2010) and capillary electrophoresis (CE) (Qin, Li, 2002; Flores *et al.*, 2004).

Likewise, vardenafil and tadalafil in different matrices were analyzed using different analytical systems, such as LC (Gratz *et al.*, 2004; Ramakrishna *et al.*, 2004; Madhavi *et al.*, 2008; Farthing *et al.*, 2010; Lake *et al.*, 2010; Di *et al.*, 2011; Lee *et al.*, 2011; Hasegawa *et al.* 2012), gas chromatography (Man *et al.*, 2009; Papoutsis *et al.*, 2010; Strano-Rossi *et al.*, 2010) and capillary electrophoresis (Ali, Aboul-Enein *et al.*, 2004; Flores *et al.*, 2004; Idris; Alnajjar, 2007).

Accordingly, this review focuses on some currently available oral therapies for ED. A brief review of the phosphodiesterase type 5 inhibitors, mechanism of action, adverse effects, pharmacokinetics and analytical methodologies employed for the determination of four PDE5i (udenafil, lodenafil carbonate, mirodenafil and avanafil) is presented.

Physiology of erectile dysfunction and PDE5i mechanism of action

Erections are initiated, maintained, and terminated because of a complex interaction between the neural and vascular components as well as the penile vasculature (McNamara, Donatucci, 2011). Visual, auditory, and tactile erectogenic stimuli are integrated and processed centrally in several hypothalamic structures. Once integrated, both branches of the autonomic nervous system recruit nerves innervating the erectile tissues of the penis (Barret *et al.*, 2005).

The main physiologic event is the release of nitric oxide (NO) from the autonomic nerve endings and the endothelial cells in the corpus cavernosum. Release of NO leads to relaxation of smooth muscle cells in the corpora cavernosa vasculature and tumescence followed by passive veno-occlusion as the subtunical venule plexus is compressed against the rigid tunica albuginea. NO facilitates vasodilatation and relaxation by activating guanylate

cyclase (GC). GC then catalyzes the breakdown of guanosine triphosphate into 3'5'-cyclic guanosine monophosphate (cGMP), leading to smooth muscle relaxation in the blood vessels supplying the corpus cavernosum, resulting in increased blood flow and an erection (Williams, Melman, 2012). Levels of cGMP in the smooth muscle cells of the penis are regulated by the enzyme phosphodiesterase type 5 (Barret *et al.*, 2005; Albersen *et al.*, 2011; McNamara, Donatucci, 2011).

PDE5i are nonhydrolyzable analogs of cGMP and exert their beneficial effects on smooth muscle relaxation competitively binding to the catalytic site of PDE5 (Albersen *et al.*, 2011). PDE5i inhibit the degradation of cGMP by phosphodiesterase type 5, increasing blood flow to the penis during sexual stimulation (Gooren, 2008).

Adverse events with PDE5i

Adverse event profiles for all of the PDE5i are similar and are the result of the vasoactive nature of these agents, producing vasodilation in vascular beds other than the corpora cavernosa. The most common side effects reported following the use of PDE5i include headache, flushing, dyspepsia, nasal congestion (Carson, 2007; Albersen *et al.*, 2011; McNamara, Donatucci, 2011). Adverse events are generally mild in nature and self-limited by continuous use, and the dropout rate due to adverse events is similar to that seen with placebo (Andersson, 2011). As to cardiovascular safety, it has been shown that PDE5 inhibitors do not affect the ability of patients with coronary artery disease to maintain a level of exercise similar to that required for sexual activity and that patients do not experience significant side effects from the use of these medications (Uckert; Stief, 2011). Nitrates are totally contraindicated with all PDE inhibitors due to unpredictable hypotension (Carson, 2007; Andersson, 2011; Uckert, Stief, 2011).

UDENAFIL

Pharmacokinetic properties

Udenafil (5-[2-propyloxy-5-(1-methyl-2-pyrollidinylethylamidosulphonyl) phenhyl]-1-methyl-3-propyl-1,6-dihydro-7*H*-pyrazolo(4,3-d)-pyrimidin-7-one developed by Dong-A Pharmaceutical Company, Korea, is also a potent PDE5 inhibitor with a similar molecular structure to sildenafil citrate (Figure 1) (Kim *et al.*, 2008; Han *et al.*, 2010). Udenafil has been marketed in Korea since 2005 under the brand name of Zydena in 100 and 200 mg tablet strengths for the treatment of erectile dysfunction (Bae *et al.*, 2008).

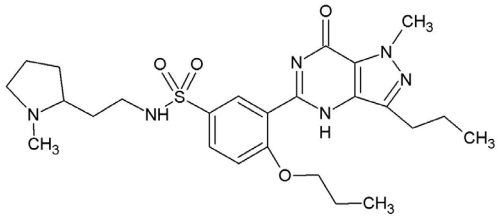

FIGURE 1 - Chemical structure of udenafil.

At a dose of 30 mg/kg, the absorption of udenafil from rat intestinal tract is known to be complete (about 99%). Thus, the absolute oral bioavailability of udenafil was 38.0% in rats (Shim *et al.*, 2003). The mean plasma concentration–time profiles of udenafil after a single oral administration of udenafil 100 mg to six healthy Korean male volunteers and the relevant pharmacokinetic parameters were determined. The mean Cmax of udenafil was 302 ± 88.5 ng/mL, Tmax 1.5 h (Kim *et al.*, 2008). The half-life and AUC values of udenafil were 11.2 ± 1.62 and 2070 ± 292 ng h/mL, respectively (Bae *et al.*, 2008).

Methods of analysis

Only a few analytical methods using liquid chromatography with ultraviolet detection and high-performance liquid chromatography coupled with tandem mass spectrometry for determination of udenafil in biological samples have been reported (Table I). No analytical method exists, so far, for the assay of udenafil in pharmaceutical formulations.

Shim *et al.* (2002) describe the LC method for the determination of udenafil in rat plasma and urine. Sample preparation is achieved using a liquid/liquid extraction procedure. It was possible to study the pharmacokinetics of udenafil in rats using the detection limits obtained. The detection limits for udenafil in rat plasma and urine were 20 and 100 ng/mL, respectively. However, the lower limit of quantification obtained in rat plasma, 20 ng/mL, was considered to have insufficient sensitivity for the determination of udenafil in human biological samples. A more sensitive assay method was developed for the quantification of udenafil in human plasma and urine (Cho *et al.*, 2003). The method was based on a modification of the method described by Shim *et al.* (2002). A single step liquid–liquid extraction procedure was performed and the lower limit for quantification was 5 ng/mL for plasma and 10 ng/mL for urine samples. This assay was successfully tested in clinical phase I studies in healthy volunteers.

Next, Kim *et al.* (2003a) described a LC/MS/MS method using liquid–liquid extraction for the determination of udenafil in human plasma. The method showed

TABLE I - Parameters described in the literature to determine udenafil using liquid chromatography

Reference	Sample	Column	Mobile phase/flow/gradient	Detector
Shim *et al.* (2002)	rat plasma and urine	C18 column (150 mm x 4.6 mm, i.d.; 5 µm particle size; Hichrom HPRPB, Berkshire, UK)	20 mM KH_2PO_4 (pH 4.7):acetonitrile (70:30, v/v for plasma and 75:25, v/v for urine Samples/1.0 mL/min /isocratic	UV
Cho *et al.* (2003)	human plasma and urine	C18 column (150 mm × 4.6 mm i.d.; 5 µm particle size; Shiseido, Tokyo, Japan)	30% acetonitrile:70% 20mM potassium phosphate buffer (pH 4.5) /1.0 mL/min/isocratic	UV
Kim *et al.* (2003b)	human plasma	luna phenylhexyl column (100 mm x 2 mm; i.d. 3 µm particle size; Phenomenex, Torrance, CA, USA)	acetonitrile:ammonium formate (5mM, pH 6.0) (60:40, v/v)/ 0.2 mL/min/isocratic	MS/MS
Kim *et al.* (2008)	plasma and urine	C18 column (150 x 4.6 mm i.d.; 5 µm particle size; Shiseido, Tokyo, Japan)	acetonitrile:20 mM potassium phosphate buffer of pH 4.5 (30:70%, v/v)/ 1.0 mL/min, isocratic	UV
Ku *et al.* (2011)	rat plasma	C18 column (50 mm x 2.1 mm; i.d., 3µm particle size; Varian Inc., CA, USA)	acetonitrile:10 mM ammonium acetate (90 : 10, v/v)/0.2 mL/min/isocratic	MS/MS

sensitivity (2.0 ng/mL) and the suitability of the method was confirmed in the pharmacokinetic study of udenafil in man.

Kim *et al.* (2008) conducted the first-in-human clinical trial to evaluate the safety, tolerability and pharmacokinetic characteristics after single and multiple oral administrations in healthy male subjects. The determination of udenafil concentrations in plasma and urine were performed using high-performance liquid chromatography, as described by Shim *et al.* (2002), with slight modifications.

According to Bae *et al.* (2008) the reported methods required time-consuming and laborious extraction procedures after sample alkalization (Shim *et al.*, 2002; Cho *et al.*, 2003; Kim *et al.*, 2003b), or a relatively large sample volume of 1 mL (Cho *et al.*, 2003) and also long chromatographic run times (Shim *et al.*, 2002; Cho *et al.*, 2003; Kim *et al.*, 2003b), which lower sample throughput capacity and sensitivity. In order to solve these problems Bae *et al.* (2008) proposed a rapid, sensitive, simple and accurate method for simultaneous determination of udenafil and its active metabolite in human plasma and urine using ultraperformance liquid chromatography coupled to tandem mass spectrometry (UPLC/MS/MS) with direct injection after simple protein precipitation. UPLC separation was achieved using an Acquity™ UPLC BEH C18 column (50 x 2.1 mm, i.d.; particle size, 1.7 µm; Waters, Milford, MA, USA). The isocratic mobile phase consisting in acetonitrile and 0.1% formic acid (75:25, v/v), the flow rate 0.4 mL/min and total run time less than 1 min. The concentrations of udenafil and its active metabolite in human plasma or urine were quantified using a Quattro Premier™ XE tandem quadrupole mass spectrometer (Waters, Milford, MA, USA) equipped with an electrospray ionization interface used to generate positive ions $[M + H]^+$. This method was successfully applied to a pharmacokinetic study of udenafil 100 mg in healthy Korean male volunteers.

Ku *et al.* (2011) described a more sensitive and rapid analysis of udenafil in rat plasma, using an LC-MS/MS system, aiming to replace the previous methods. The authors justify their work due to the large plasma volume used, long retention times and because pharmacokinetic studies in animal model had to be conducted at high doses considering the low limit of quantification of the methods previously published (Shim *et al.*, 2002; Cho *et al.*, 2003; Kim *et al.*, 2003b, Bae *et al.*, 2008). The method resulted in a low limit of quantification (0.5 ng/mL), low plasma volume (0.1 mL) and relatively short running time (2.5 min). They intended this method to be used for the analysis of drug concentration in plasma after intravenous, intranasal and oral administrations in rats.

LODENAFIL

Pharmacokinetics properties

Lodenafil carbonate, *bis*-(2-{4-[4-ethoxy-3-(1-methyl-7-oxo-3-propyl-6,7-dihydro-1*H*-pyrazolo[4,3-d]pyrimidin-5-yl)-benzenesulfonyl]piperazin-1-yl}-ethyl)

(Figure 2) has a unique chemical structure; a carbonate bridge unites 2 molecules of lodenafil. After ingestion the carbonate bridge is broken freeing each molecule of lodenafil for biologic effect (Glina *et al.*, 2010; Toque *et al.*,

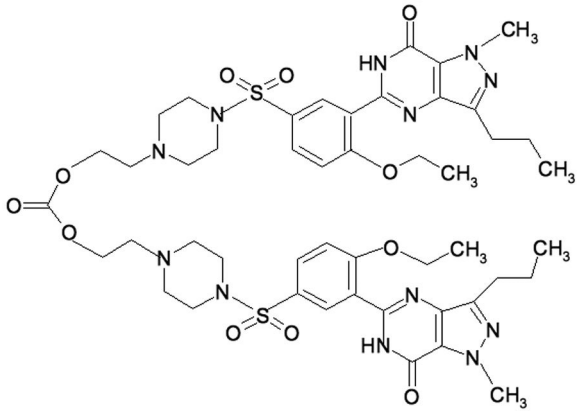

FIGURE 2 - Chemical structure of lodenafil carbonate.

The inhibition of cGMP hydrolysis by sildenafil, lodenafil or lodenafil carbonate was determined at various concentrations, and the results showed that lodenafil carbonate was more potent than lodenafil or sildenafil to inhibit PDE5 in human platelets. Lodenafil carbonate was completely metabolized into lodenafil by rat plasma. Incubation of lodenafil carbonate with human plasma and dog plasma demonstrated that a small proportion of lodenafil carbonate was metabolized to lodenafil and to an unknown metabolite (Toque *et al.*, 2008).

After oral intake of 160 mg under fasting condition, Cmax was 157 ng/mL, Tmax was 1.2 h, T1/2 was 2.4 h and AUC was 530 ng h/mL. For oral administration with a 600 kcal lipid meal, the parameters were: Cmax = 148 ng/mL; Tmax = 3.1 h; T1/2 = 2.63 h; AUC = 683 ng h/mL. Therefore, administration with lipids delayed the absorption but increased the bioavailability (Lucio *et al.*, 2007; Glina *et al.*, 2010).

Methods of analysis

The development of lodenafil carbonate was reported by Toque *et al.* (2008). They observed the effects of lodenafil carbonate on rabbit and human corpus cavernosum relaxation, activity of PDE5 in human platelets, stability and metabolic studies in comparison with sildenafil and lodenafil, as well as the pharmacological evaluation of lodenafil carbonate after intravenous and oral administration in male beagles.

The determination of PDE activity, stability of lodenafil carbonate in human, dog and rat plasma and the pharmacokinetic parameters after a single intravenous or oral dose was carried out by LC-MS/MS analysis (Table II).

Codevilla *et al.* (2011a) developed a stability-indicating reversed-phase liquid chromatography method using ultraviolet (UV) detection for the quantitative determination of lodenafil carbonate in tablets. The method can be useful for routine quality control assay and stability studies.

Another study for the determination of lodenafil carbonate in tablets was developed by Codevilla *et al.* (2011b). As an alternative to the LC method the authors suggested a UV-spectrophotometric method for the analysis of lodenafil carbonate in pharmaceutical form. The UV method offers advantages over other analytical methods due to its rapidity, simplicity, and lower cost. Recently, Codevilla *et al.* (2012) developed and validated a capillary zone electrophoresis (CZE) method for determination of lodenafil carbonate in drug products. There are some advantages to use the CZE method, such as rapid analysis, small sample and reagent consumption, high separation efficiency (Furlanetto *et al.*, 2001; Yang *et al.*, 2010). The results obtained from the UV-spectrophotometric method and CZE method were compared statistically with the LC method (Codevilla *et al.*, 2011a) and the results showed no significant difference between these methods.

TABLE II - Parameters described in the literature to determine lodenafil carbonate using liquid chromatography

Reference	Sample	Column	Mobile phase/flow/gradient	Detector
Toque *et al.*, 2009	rat, dog and human plasma	C18 column (150 mm x 4.6 mm i.d.; 4 µm particle size; Phenomenex, Torrance, CA, USA)	A=H2O-ammonium acetate 50 mM; B=CH3CN-ammonium acetate 50 mM)/ 1.5 mL/min/gradient	MS/MS
Codevilla *et al.*, 2011a	tablets	C18 column (250 mm x 4.6 mm i.d.; 4 µm particle size; Phenomenex, Torrance, CA, USA)	Methanol:acetic acid 0.1%, pH 4.0 (65:35, v/v)/1.0 mL/min/isocratic	UV

MIRODENAFIL

Pharmacokinetic properties

Mirodenafil, 5-ethyl-2-f-5-[4-(2-hydroxyethyl) piperazine-1-sulfonyl]-2-phenylg -7-propoxypropyl-3,5-dihydropyrrolo-[3,2-d]-pyrimidin-4-one (Figure 3), is a new PDE-5 inhibitor that came into the market recently (Choi *et al.*, 2009; Lee *et al.*, 2009).

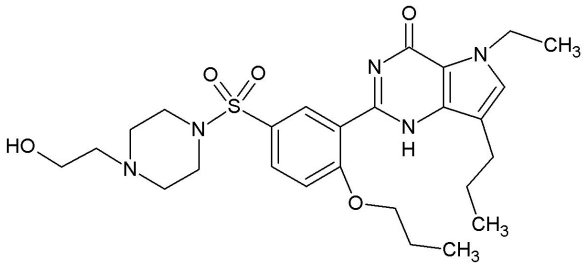

FIGURE 3 - Chemical structure of mirodenafil.

Promising animal studies showed that the maximum concentration of mirodenafil is higher than the one of sildenafil in plasma and corpus cavernosum tissue (McNamara, Donatucci, 2011). The pharmacokinetic parameters in plasma and corpus cavernosum are defined after a single oral administration. The Cmax and AUC of mirodenafil in plasma were 2728 ng/mL and 5702 ng h/mL, respectively. The Tmax was 1.0 h and the half-life was 1.5 h. In the corpus cavernosum the Cmax of mirodenafil was 2812 ng/mL, AUC 8425 ng h/mL, Tmax 1.4 h and the half-life 1.3 h (Lee *et al.*, 2009).

METHODS OF ANALYSIS

Two methods were published for the determination of mirodenafil in biological fluids. Choi *et al.* (2009) describe an isocratic reversed-phase liquid chromatographic method for simultaneous analysis of mirodenafil and its two main metabolites, SK3541 and SK3544, in rat plasma, urine, and tissue homogenates. The authors used a simple deproteinization procedure for sample preparation, and the compounds were separated on a C18 column (250 mm x 4.6 mm, i.d.; 5 µm particle size; Shiseido, Tokyo, Japan). The mobile phase was constituted with 0.02 M ammonium acetate buffer (pH 6):acetonitrile (52:48, v/v) at a flow rate of 1.4 mL/min. UV detection was at 254 nm.

Lee *et al.* (2009) developed a study with the proposed method to determine sildenafil and mirodenafil in the plasma and corpus cavernosum tissue of rats using LC–MS/MS. A CapcellPak phenyl column (2.1mm x 150 mm, 5 µm) maintained constant at 40 °C was used for the separation. The mobile phase consisted of 90% acetonitrile in 5 mM ammonium formate (pH 6.0). A gradient program was used for the LC separation with a flow rate of 0.2 mL/min.

AVANAFIL

Pharmacokinetic properties

Avanafil (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 4) is a selective PDE5 inhibitor developed by Mitsubishi Tanabe Pharma Corporation (Osaka, Japan) (Jung *et al.,* 2010; Alwaal *et al.*, 2011).

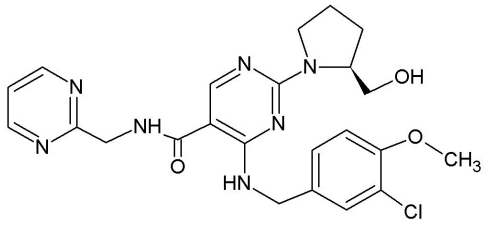

FIGURE 4. Chemical structure of avanafil.

VIVUS Inc. is in the process of developing avanafil, a fast-acting, highly selective PDE5i, as an oral medication for the treatment of ED. Avanafil was generally well tolerated and had linear pharmacokinetic properties at daily doses of 50 to 200 mg over 7 days in healthy Korean male volunteers (Uckert, Stief, 2011). Avanafil oral tablets will be available in 50, 100, and 200 mg (Traynor, 2012). A mean Tmax was reached at 0.33 to 0.52 h after oral dosing and then declined with a mean apparent T1/2 of 5.36 to 10.66 h. AUC and Cmax in a single-dose were 2217 ng h/mL and 1206 ng/mL, respectively (Jung *et al.*, 2010). A randomized, double-blind, placebo-controlled Phase III study evaluating two doses of avanafil (100 and 200 mg) in 390 men with both diabetes and ED, showed satisfactory results. FDA requires Vivus Inc. to perform two post-marketing clinical trials, in order to observe possible adverse events associated with the use of avanafil (Traynor, 2012).

Methods of analysis

Recently avanafil has gone through the phase I, II and III clinical trials and this could be the reason for few publications about methods of analysis of this drug. Jung

et al. (2010) developed a study to meet Korean regulatory requirements for the marketing of avanafil. They observed the tolerability and pharmacokinetic properties of single and multiple oral doses of avanafil in healthy Korean male volunteers. The plasma concentrations of avanafil were measured by a sensitive and selective method using on-line solid-phase extraction (SPE) coupled to LC-MS/MS. Chromatographic separation was conducted using a C18 column (50 mm x 2.0 i.d.; 3 µm particle size; Shiseido, Tokyo, Japan). The mobile phase consisted of a mixture of 10 mM ammonium formate (pH 2.5) and acetonitrile (65:35, v/v), with a flow rate of 0.3 mL/min.

CONCLUSION

Phosphodiesterase type 5 inhibitors are currently the therapeutic option of choice for erectile dysfunction. This review highlighted the mechanism of action of the PDE5i, some adverse effects, pharmacokinetic and analytical methods for the determination of four phosphodiesterase type 5 inhibitors in different matrices. As shown, there are few reports for the new PDE5i, udenafil, lodenafil carbonate, mirodenafil and avanafil, especially concerning the determinations of these drugs. Hence, in this paper we compiled applied methods, such as LC-UV, LC-MS and LC-MS/MS, in biological matrices and pharmaceutical formulations. For each of the compiled methods positive and negative features can be considered because some of them are simpler and of lower cost, but result in not so much informations, whereas others result in many informations about the drug, but need especial instrumentation and are very expensive. With the application of these methodologies quality control routine will be possible, assuring drug safety. A major feature of this paper is the compilation of information about udenafil, lodenafil carbonate, mirodenafil and avanafil in the same paper.

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