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Sildenafil Determination in Various Matrices: A Review

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ABSTRACT

Determination of sildenafil by various methods and in various media is reviewed in this paper. The methods used consist of ultraviolet (UV) spectroscopy, thin layer chromatography (TLC), and high-performance liquid chromatography (HPLC). These methods were used to determine the amount of sildenafil in pharmaceutical preparations, soft drinks, and biological liquids. HPLC was used for evaluation of pharmacokinetic parameters of sildenafil. It is concluded that HPLC is the most reliable and applicable method in this regard.

Keywords: *Sildenafil,* HPLC, pharmaceutical preparations, soft drinks, biological liquids

Male erectile dysfunction has been defined as the inability to attain and/or maintain penile erection sufficient for satisfactory sexual performance [1]. A recent and extensive population-based survey confirmed the high prevalence of this disorder, with an estimated prevalence across all ages of 10%, rising to 52% in men aged between 50 and 70 years [2]. Male erectile dysfunction is generally accepted to affect adversely the quality of life and there is evidence to indicate that it is frequently associated with depression, increased anxiety and poor self-esteem in affected patients [1-4]. Although male erectile dysfunction represents a major clinical problem, medical therapy for this condition remains unsatisfactory because it was invasive or ineffective before the introduction of sildenafil [6].

Recent studies that have examined the mechanism of penile erection have indicated that relaxation of the corpus cavernosal smooth muscle cells, which is mediated by both non-adrenergic, non-cholinergic neurons and by cholinergic mechanisms, is caused by nitric oxide and its second messenger, cyclic guanosine monophophate (cGMP) [4, 5]. Slidenatil (5-[2-ethoxy-5-(4-methylpiperazin-1-ylsulfonyl) phenyl]-1-methyl-3 propyl-1, 6-dihydro-7H-pyrazolo[4, 3-d] pyrimidin-7-one), Fig 1 [6], is a potent and competitive inhibitor of the type-V cGMP specific phosphodiesterase enzyme, the predominant isoenzyme in the human corpus cavernosum. As such, sildenafil enhances relaxation of the corpus cavernosal smooth muscle, which in turn increases blood flow into the cavernosal spaces, thus leading to increased intracavernosal pressure, a key factor in producing an erect penis [7, 8]. However, the introduction of sildenafil resulted to its widespread use as well as its abuse. Therefore, specific, accurate, and robust determination of this drug is widely required. Several methods have been developed for this purpose. This review attempts to summarize these works.

UV SPECTROSCOPIC DETERMINATION

UV spectrophotometric method [9] at 292 nm was used for determination of sildenafil citrate in pharmaceutical preparations and its value was compared with flow injection (FIA) method. A higher mean and lower relative standard deviation percent (RSD %) and better clearance (CL) values were found for spectrophotometeric method.

TLC METHODS

TLC methods are developed for identification of sildenafil as an illegal additive in roborant soft drinks and foods [10, 11]. The sample dissolved for TLC was applied to silica gel 60 F_{254} plates (Merk, Darmstadt, Germany) with (A) chloroform/ammonia solution/methanol (15:3:2, lower layer) and (B) chloroform / diethylamine/methanol (15:3:2) as the developing solvent. Spots were located under UV radiation at 254 nm and sprayed evenly with Dragendorff's regent [10]. After development, dark blue spots of sildenafil were observed and under UV light (mainly 254 nm wavelength), which were clearly resolved and well separated from the other components. In addition, Dragendorff's reagent was sprayed evenly on the plates and yellow-red

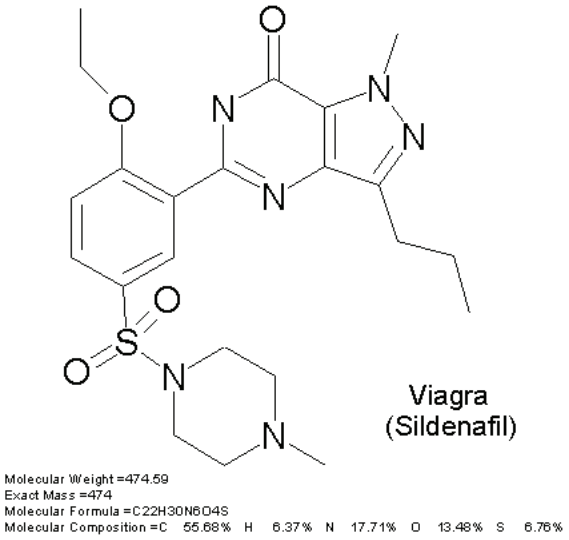

Fig 1. The structure of sildenafil.

spots were visualized. Direct UV measurements were more sensitive than visualized measurements for sildenafil. With solvent, (A) RF value of sildenafil was 0.7. With solvent (B) the RF value of sildenafil was 0.8. The UV measurement allows reliable identification up to 0.15 μg and provides good resolution and separation [10]. Sildenafil in heath foods could be extracted with ethyl acetate under alkaline conditions as sample solutions for TLC. The sample solution for TLC was applied to Silica gel F_{254} plates with chloroform/methanol/28% ammonia (90:1:5, under layer) as mobile phase. Spots were located under UV radiation at 254 nm and 366 nm, and sprayed with Dragendorff reagent. When this was applied to commercial heath foods, sildenafil could be identified and detected up to 25-45 mg/tablet or bottle. Therefore, there is a fear of side effects for Sildenafil, when it is taken as heath foods.

HPLC Methods

In formulation products and dosage forms. HPLC was found to be a useful instrument for determination of sildenafil and related compounds in pharmaceutical preparations [12, 13]. They [12] developed a specific, precise, accurate, reproducible and robust method using C18 column and an UV detector (set at 240 nm) for good isocratic chromatographic separation for sildenafil and other related substances, impurities which may originate from synthesis process or degradation. Mobile phase used was a mixture of ammonium acetate (pH 7.0, 0.2 M)-acetonitil (1:1 v/v) and the flow rate was 1 mL/min. The relative standard deviation (R.S.D.) of six replicate injections of standard preparation was not greater than 2.0% and tailing factor was less than 3.0. The specificity was demonstrated by induced degradation of sildenafil citrate samples by treating them with either 1.5 % H_2O_2 and storing the sample at room temperature for 65 Min, 0.1 HCl M and storing at 65°C for 12 days, or 0.1 M NaOH, and storing at 65°C for 12 days or by heating a pure solid sildenafil citrate sample at the melting point for 5 min. The recovery of sildenafil was 101.7 and 99.3 % with a total degradation of 0.2 and 0.4 % in the case of 0.1 M HCl and 0.1 M NaOH, respectively.

Similarly, RP-LC HPLC system was used for quantitation of sildenafil citrate in pure and its pharmaceutical preparations [13] using piroxicam as internal standard. They showed that this technique [13] provided a precise and accurate method for its determination in pharmaceutical formulation. Their method [13] is reproducible and it is found that the data are consistent with the label claim.

In heath food and soft drinks. Dietary supplements advertising roborant nutrition or weight reduction have gain popularity in Japan and other industrial countries in recent years. The presence of therapeutic medicinal ingredients often added to supplements as part of the intended use has been reported [11, 14]. Prolonged or excessive consumption of these supplements containing undeclared amounts of drugs may cause serious adverse heath consequences. Such practices violate relevant pharmaceutical and medical laws and are subject to judicial prosecution or severe administrative penalties. For periodic inspection, the establishment of more effective identification/determination procedure for screening medicinal ingredient in supplements is required [15, 16]. Sildenafil citrate is the first effective oral treatment for erectile dysfunction of various etiologies [6]. Its use is absolutely contraindicated with patients receiving nitrate therapy because of the potential for significant hypotensive effects [17, 18]. HPLC system with u.v. [11], photo-diode, and mass spectrometric detectors [10], were used for quantitative analysis of sildenafil in health foods and soft drinks. Sildenafil in health foods was extracted with 50% methanol and then diluted with HPLC mobile phase as sample solution for HPLC. The HPLC analysis was carried out on a column of Cosmosil 5C18-AR (4.6 mm × 150 mm, 5 mm) with 0.05 mol/L phosphate buffer pH 3.0/acetonitrile (73:27) as mobile phase and the elute was monitored by a photo-diode array detector. The quantitative analysis was available, when the peak of this sample on HPLC was detected at 290 nm. When this system was applied to commercial heath foods, Sildenafil was identified and its content was 25-45 mg/tablet or bottle. An easily available, simultaneous identification/determination procedure for sildenafil in soft drinks was established by using a combination of different analytical methods; TLC, LC/MS, and HPLC [10]. The HPLC analysis was perfotmed on a Waksil 5C18 (4.6 mm × 150 mm, 5 μm) column with water/methanol/acetonitril/triethylamin (580:250:170:1) adjusted with phosphoric acid to pH 3.0 as the mobile phase, and the effluent was monitored with a photo-diode-array detector. Quantitative HPLC analysis of sildenafil was carried out at 280 nm. When this procedure was applied to commercial soft drinks, sildenafil was identified and determined at a concentration of 44mg per bottle. These contents nearly correspond to that in Viagra, 25 mg/tablet, Therefore is a fear of side effects for Sildenafil, when it is taken as health foods [11] or soft drinks [10].

Determination of Sildenafil in Biological Samples. One mL of plasma sample were extracted in one-step liquid-liquid extraction after alkaline treatment and analysis by HPLC equipped with Inertsil 5 ODS-2 column (with a particle size of 5 μm) and a UV detector [19]. UV detection was carried at 230 nm. Mobil phase consisted of acetonitrile and 30-mM potassium dihydrogen phosphate buffer solution (pH 6.0 adjusted with 1 N NaOH) at a 55:45 (v/v) ratio. The flow-rate was set at 0.5 mL/min. The method was found to be selective, precise, and linear over a concentration range of 10-1000 ng/mL [19]. This method was applied in bioavailability studies of sildenafil and study of the influence of the co-administration of grapefruit on silsenafil pharmacokinetics [19].

Similarly, sildenafil concentration in a postmortem blood samples was measured by HPLC equipped with MS detector and found to be in the range of 6.27 μg/mL [20].

In another work, a simple and sensitive high-performance liquid chromatographic method was described [21] for the determination of sildenafil transdermal permeation of nude mouse skin. A reversed-phase column with UV detection at 224 nm was used for chromatographic separation. The mobil phase consisted of 32% acetonitril with 0.2% phosphoric acid in water at pH 5.3 adjusted with 10 M NaOH with the flow-rate set at 1.0 mL/min. The limit of quantitation achieved was 5 ng/mL, and the calibration curve showed good linearity over the concentration range of 5-500 ng/mL. The relative standard deviations of within- and between-day analysis were all within 15%. Sildenafil was found to be stable between pH 3 and 12 during 24 –h incubation with skin. After transdermal administration of 15.8 μg/mL of sildenafil to nude mouse skin, it was detected as early as 15 min. The transport amount of sildenafil could be quntitated and, at pH 8-11, had the highest permeation rate in nude mouse skin.

HPLC Application in Pharmacokinetics Studies

HPLC was found to be the most suitable method for application in Pharmacokinetics studies Sheu et al. describe [22] a simple HPLC system capable for eluting and extracting sildenafil from human plasma which complies with general requirements for system suitability. Preliminary studies were directed towards the effect of certain variables on the suitability of the method. The parameters assessed include the type and quantity of the organic modifires, the column, the salt concentration, and the pH of the mobile phase. The results show that the Inertsil 5 ODS-3 column was suitable for the determination of sildenafil from human plasma because of its excellent resolution, appropriate retention time, and good sensitivity. It was found that their method which uses one-step liquid-liquid extraction of sildenfil from plasma after alkaline treatment was satisfactory. It was that the recovery of sildenafil added to human plasma was almost quantitative (at least 76%) with minimal interference. It is found that the method is reproducible and suitable for the analysis of plasma samples used in studies of sildenafil pharmacokinetics. In a similar study the HPLC method is used in the study of sildenafil pharmacokinetics and interaction of grape fruit juice on its pharmacokinetic [23] Lee and. Min [23] found that sildenafil reached a peak height of 1067.7 ng/mL in one hour post administration of 100 mg of sildenafil tablet to a 72 year old male and declined with half-life of 4.9 hour. The Cmax of drug will be reduces to 1517.0 ng/mL and its half life will be increase to 5.3 hours in the presences of grip fruit.

CONCLUSION

It has been found that HPLC method is the most used and reliable way for the determination of sildenafil in various samples, such as pharmaceutical preparations, soft drinks, serum and other biological fluids, and to determine its pharmacokinetics parameters.

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