EUROPEAN JOURNAL OF BIOMEDICAL AND PHARMACEUTICAL SCIENCES
http://www.ejbps.com

ISSN 2349-8870
Volume: 1
Issue: 3
147 - 156
Year: 2014

ANALYTICAL METHOD DEVELOPMENT AND VALIDATION FOR THE ESTIMATION OF UDENAFIL IN BULK AND PHARMACEUTICAL DOSAGE FORM BY RP-HPLC

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Article Received on 12/10/2014 **Article Revised on 19/10/2014** **Article Accepted on 25/10/2014**

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ABSTRACT

A simple, sensitive and rapid reverse phase high performance liquid chromatographic method was developed for the estimation of udenafil in pure and pharmaceutical dosage forms. A Altima C_{18} column (150 x 4.6mm x 5µm) was used as a stationary phase with a mobile phase containing a mixture of buffer (accurately weighed and transferred 1.36gm of Potassium dihydrogen orthophosphate in a 1000ml of volumetric flask add about 900ml of milli-Q water added add 1ml of triethylamine and degass to sonicate and finally make up the volumewith water, then pH adjusted to 3.1 with dil. Ortho phosphoric acid solution) and acetonitrile in the ratio of 60:40v/v. The flow rate was 1.0ml/min, the effluent was monitored at 246nm and eluted at 2.811min. Calibration curve was plotted with a range from 25-150µg/ml for udenafil and the correlation was found to be 0.9999.The accuracy range was found between 99.48% and 101.54%. The %RSD values for both intraday and interday precision were less than 1%. The limit of detection (LOD) and limit of quantification (LOQ) were found to be 1.015µg/ml and 3.075µg/ml respectively. The assay was validated for the parameters like specificity, system suitability, precision, accuracy, robustness and ruggedness parameters. The proposed method can be useful for the routine determination of udenafil in pharmaceutical dosage form.

KEYWORDS: Udenafil, Calibration curve, RP-HPLC, Validation.

INTRODUCTION

Udenafil, chemically it is 3-{1-methyl-7-oxo-3-propyl-1H,4H,7H-pyrazolo[4,3-d]pyrimidin-5-yl}-N-[2-(1-methylpyrrolidin-2-yl)ethyl]-4-propoxybenzene-1-sulfonamide. The chemical formula is $C_{25}H_{36}N_6O_4S$. Udenafil is a drug used in urology to treat erectile dysfunction. It belongs to a class of drugs called PDE5 inhibitor, which many other erectile dysfunction drugs such as sildenafil, tadalafil, and vardenafil also belong to. It was developed by Dong-A Pharmaceutical Co., Ltd. and is marketed under the trade name Zydena. With a T max of 1.0-1.5 h and a T 1/2 of 11-13 h (a relatively rapid onset and a long duration of action), both on-demand and once-daily use of udenafil have been reported. Typical doses are 100 and 200 mg. Udenafil is available in Korea, Russia, and Philippines in the United States, it is not approved for use U.S. Food and Drug Administration.

Udenafil inhibits the cGMP specific phosphodiesterase type 5 (PDE5) which is responsible for degradation of cGMP in the corpus cavernosum located around the penis. Penile erection during sexual stimulation is caused by increased penile blood flow resulting from the relaxation of penile arteries and corpus cavernosal smooth muscle. This response is mediated by the release of nitric oxide (NO) from nerve terminals and endothelial cells, which stimulates the synthesis of cGMP in smooth muscle cells. Cyclic GMP causes smooth muscle relaxation and increased blood flow into the corpus cavernosum. The inhibition of phosphodiesterase type 5 (PDE5) by udenafil enhances erectile function by increasing the amount of cGMP[1,2].

Literature surveys reveal few methods for its determination[3-10]. The simple, accurate, precise and validated method for determination of udenafil was developed by RP-HPLC method.

EXPERIMENTAL

Reagents

Udenafil was kindly supplied by Cadila Healthcare Ltd. Acetonitrile, water (HPLC grade, Merck) and all the other reagents of AR grade were purchased from M R Enterprisers. A tablet UDZIRE (manufactured by Dong-A Pharmaceutical Co. Ltd and imported by Cadila Healthcare Ltd) containing 100mg of udenafil.

Instrumentation

The LC system consisted of a Waters model 515, PDA detector 2998 with 20 µL sample loop. The output signals were monitored and integrated using Empower 2 software.

Chromatographic conditions

The elution was isocratic and the mobile phase consisted of a mixture of buffer and acetonitrile (60: 40 v/v). The mobile phase was filtered through a 0.45-µm (HVLP, Germany) membrane filter prior to use. A Altima (150 x 4.6mm x 5 µ) was used for determination. The flow rate was 1.0 ml/min and the column was operated at ambient temperature (~30 ^{o}C). The volume of sample injected was 10 µL. Prior to injection of the solutions, column was equilibrated for at least 30min with mobile phase flowing through the system. The UV detector was set at wavelength of 246nm. A typical chromatogram of udenafil is shown in (Fig. 2).

Diluent: Acetonitrile and Water (50:50)v/v

Standard Preparation

Stock solution of Udenafil was prepared by dissolving 100mg in 100 ml volumetric flask add few ml of diluent. Sonicate it for 30min and make up with diluent. Transfer 1ml from the above solution into 10ml volumetric flask to get concentration of 100µg/ml.

Sample Preparation

About 20 tablets were taken and their average weight was calculated. The tablets were crushed to a fine powder and drug equivalent to 100mg was transferred to a 100 ml volumetric flask, dissolved in diluents. Transfer 1ml from the above solution into 10ml volumetric flask and filtered through 0.45µ membrane filter to get concentration of 100µg/ml.

METHOD VALIDATION

The developed method was validated as per ICH guidelines [11-12] for its accuracy, linearity, precision, specificity, robustness, ruggedness, limit of detection and limit of quantification by using the following procedures. The parameters are validated as shown in table 15.

System suitability

System suitability and chromatographic parameters were validated such as asymmetry factor, tailing factor and number of theoretical plates were calculated (Figure: 1).

Linearity

Linearity of this method was evaluated by linear regression analysis and calculated by least square method and studied by preparing standard solutions of udenafil at different

concentration levels. Absorbance of resulting solutions was measured and the calibration curve was plotted between absorbance vs concentration of the drug (Figure: 2). The response was found to be linear in the range 25-150µg/ml for udenafil. The data was given in table 1.

Accuracy

Accuracy was performed in triplicate for various concentrations of udenafil equivalent to 50%, 100% and 150% of the standard amount were injected into the HPLC system per the test procedure. The average % recovery was calculated. The data was given in table 2.

Precision

A) Method Repeatability

Six sample solutions of the same concentration (100%) were prepared and injected into the HPLC system as per test procedure. The results were given in table 3.

B) Intermediate Precision (Day to Day variability)

Two days as per test method conducted the study. For Day-1 and Day-2, six sample solutions of the same concentration (100%) were prepared and injected into the HPLC system as per test procedure. The results were given in table 6.

Limit of detection and Limit of Quantification

LOD and LOQ were calculated from the average slope and standard deviation from the calibration curve as per ICH guidelines. LOD and LOQ were found to be 1.015µg/ml and 3.075µg/ml respectively.

Robustness and Ruggedness

Robustness was done by small deliberate changes in the chromatographic conditions and retention time of udenafil was noted. The factors selected were flow rate and variation in the mobile phase composition. The results remained unaffected by small variations in these parameters as shown in table 4 and 5. Ruggedness of the method was checked by using different days and instruments. The relative standard deviation of the results obtained from different days and instruments was <2.0%. The results were given in table 6 and 7.

Assay

The assay & % purity was performed by taking brand UDZIRE with label claim 100mg. The observed value was compared with that of standard value without interference from the excipients used in the tablet dosage form. The results were given in table 8.

Degradation studies

The standard solution injected in the chromatographic system and the standard chromatogram was compared with different stress degradation conditions chromatogram. The results were given in table 9, 10, 11, 12, 13&14.

RESULTS

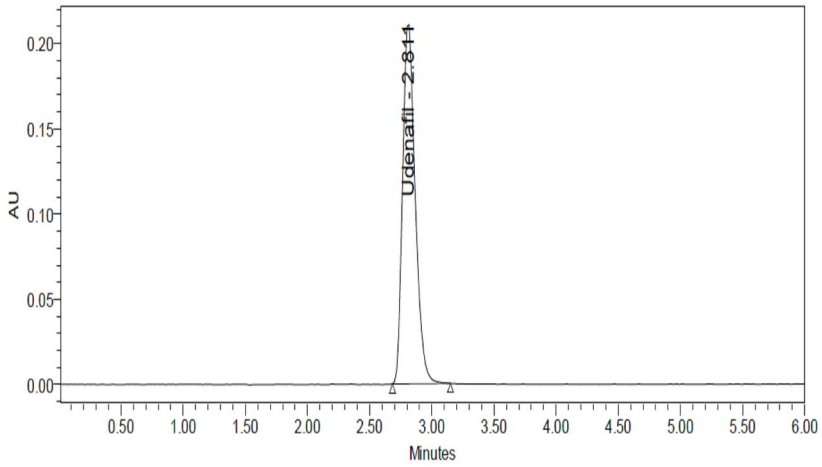

Fig - 1: HPLC Chromatogram of Udenafil

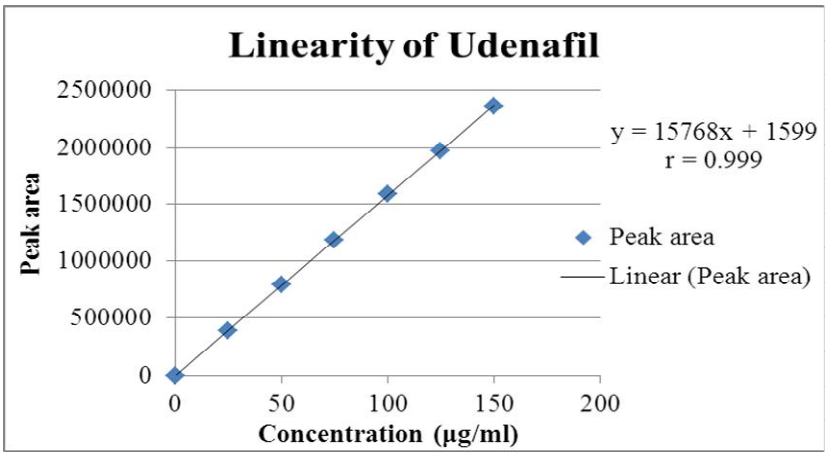

Fig – 2: Linearity of Udenafil in the range 25 to 150µg/ml.

Table 1: Linearity data of Udenafil

S.No	Concentration (µg/ml)	Injection	Retention time (mins)	Area
1	25	1	2.827	390136
2	50	1	2.81	793523
3	75	1	2.753	1182415
4	100	1	2.811	1591981
5	125	1	2.744	1974592
6	150	1	2.818	2356741
R = 0.9999				
y = 15768x + 1599				

Table 2: Accuracy data

S.No.	Spiked level	Amount Added(µg/ml)	Amount Found(µg/ml)	Average %Recovery*	Std.Dev	%RSD
1(n=3)	50%	25	24.91	99.62	0.05	0.05
2(n=3)	100%	50	50.56	101.12	0.38	0.38
3(n=3)	150%	75	74.89	99.86	0.33	0.33

*n=3 (Average of 3 determinations)

Table 3: Precision data of 100µg/ml

S.No.	Concentration(µg/ml)	Injection	Retention time (mins)	Area
1	100	1	2.828	1509457
2	100	1	2.84	1499699
3	100	1	2.842	1498727
4	100	1	2.847	1499143
5	100	1	2.85	1508017
6	100	1	2.851	1500187
Mean				1502538
Std.Dev				4848
%RSD				0.32

Table 4: Robustness data relating to change in flow rate (1.0ml/min)

S.No	Flow rate (ml/min)	Average Peak Area*	SD	%RSD
1	0.9ml/min	1502466	5832	0.39
2	1.0ml/min	1502411	4861	0.32
3	1.1ml/min	1501465	3825	0.25

*n=3 (Average of 3 determinations)

Table 5: Robustness data relating to change in mobile phase composition

S.No	Mobile Phase Variation (%)	Average Peak Area*	SD	%RSD
1	M.P-1-(ACN:H_2O::61:39)	1501452	4217	0.28
2	M.P-2-(ACN:H_2O::60:40)	1502919	3307	0.22
3	M.P-3-(ACN:H_2O::59:41)	1500950	3800	0.25

*n=3 (Average of 3 determinations)

Table 6: Ruggedness data relating to change of day

S.No	Inter-day Precision		
	Peak Area		
	Concentration (µg/ml)	Day – 1	Day – 2
1	100	1504147	1506847
2	100	1498474	1499283
3	100	1499283	1498391
4	100	1498511	1499037
5	100	1505833	1503821
6	100	1501837	1505382

Mean		1501348	1502127
SD		3123	3670
%RSD		0.21	0.24

Table 7: Ruggedness data relating to change of instrument

S.No	Instrument to Instrument		
	Peak Area		
	Concentration (µg/ml)	Instrument – 1	Instrument – 2
1	100	1502038	1499409
2	100	1506822	1499333
3	100	1497292	1508473
4	100	1498292	1504859
5	100	1499038	1509473
6	100	1501038	1498336
Mean		1500753	1503314
SD		3451	4956
%RSD		0.23	0.33

Table-8: Results of analysis of laboratory samples (Assay)

Sample	Label	Amount found	% Purity ± RSD*
Brand-1 (UDZIRE)	100mg	100.08mg	100.03 ± 0.12

*n=3 (Average of 3 determinations)

Table-9: Results of acid degradation studies of udenafil

S.No	Udenafil				
	Concentration(µg/ml)	Time(hrs)	Area	%Assay	%Degradation
1	100	0	1497636	100	
2	100	24	1390048	92.77	-8

Table-10: Results of base degradation studies of udenafil

S.No	Udenafil				
	Concentration(µg/ml)	Time(hrs)	Area	%Assay	%Degradation
1	100	0	1497636	100	
2	100	24	1406536	93.87	-8

Table-11: Results of peroxide degradation studies of udenafil

S.No	Udenafil				
	Concentration(µg/ml)	Time(hrs)	Area	%Assay	%Degradation
1	100	0	1497636	100	
2	100	24	1421094	94.84	-6

Table-12: Results of thermal degradation studies of udenafil

S.No	Udenafil				
	Concentration(µg/ml)	Time(hrs)	Area	%Assay	%Degradation
1	100	0	1497636	100	

2	100	24	1434604	95.74	-5

Table-13: Results of UV degradation studies of udenafil

S.No	Udenafil Concentration(µg/ml)	Time(hrs)	Area	%Assay	%Degradation
1	100	0	1497636	100	
2	100	24	1476310	98.53	-2

Table-14: Results of neutral degradation studies of udenafil

S.No	Udenafil Concentration(µg/ml)	Time(hrs)	Area	%Assay	%Degradation
1	100	0	1497636	100	
2	100	24	1497594	99.95	-0.05

Table-15: System suitability parameters

Validation parameters	Results
Linearity range (µg/ml)	25 – 150
Regression equation	Y = 15768x + 1599
Correlation Coefficient(r^2)	0.9999
Accuracy	99.48% to 101.54%
Precision (%RSD)	0.32
Robustness (%RSD)	
Flow rate (0.9ml/min & 1.1ml/min)	NMT 0.39
Mobile phase – ACN : H_2O(61:39 & 59:41)	NMT 0.28
Ruggedness (%RSD)	
Interday – (Day 1 & Day 2)	NMT 0.24
Instrument to Instrument (Inst-1 & Inst-2)	NMT 0.33

A reverse-phase column procedure was proposed as a suitable method for the determination of udenafil dosage form. The chromatographic conditions were optimized by changing the mobile phase composition. Different ratios were experimented to optimize the mobile phase. Finally, buffer and acetonitrile in the ratio 60:40v/v was used as mobile phase, which showed good resolution of Udenafil peak. The wavelength of detection selected was 246nm, as the drug showed optimized absorbance at this wavelength. By our proposed method the retention time of udenafil was about 2.811 minute and none of the impurities were interfering in its assay.

DISCUSSIONS

The statistical analysis of data and the drug recovery data showed that the method was simple, rapid, economical, sensitive, precise and accurate. It can thereby easily adopt for routine quality control analysis. The results of this analysis confirmed that the proposed

method was suitable for determination of drug in pharmaceutical formulation with virtually no interference of additives. Hence the proposed method can be successfully applied in estimation of udenafil in marketed formulation.

CONCLUSION

The proposed method is rapid, accurate and sensitive. It makes use of fewer amounts of solvents and change of set of conditions requires a short time. This method can be suitably analyzed for the routine analysis of udenafil in bulk and its tablet dosage forms. It does not suffer from any interference due to common excipients present in pharmaceutical preparation and can be conveniently adopted for quality control analysis.

ACKNOWLEDGEMENTS

The authors are thankful to Mallareddy College of pharmacy, Secunderabad for providing all facilities to complete the work and Cadila Healthcare Ltd for providing the Udenafil as gift sample.

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