World Journal of Pharmaceutical Sciences
ISSN (Print): 2321-3310; ISSN (Online): 2321-3086
Published by Atom and Cell Publishers
Available online at: http://www.wjpsonline.org/
Original Article

Method development and method validation of udenafil in bulk and pharmaceutical dosage form by UV– spectrophotometric method

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Received: 25-06-2014 / Revised: 08-07-2014 / Accepted: 20-09-2014

ABSTRACT

A simple, precise and accurate UV Spectrophotometric method has been developed for estimation of udenafil in bulk and tablet dosage form. In this method udenafil shows λmax at 291nm using 0.1N HCl as a solvent. The responses were linear in the range of 5-35µg/ml. The regression equation of the calibration graph and correlation coefficient were found to be y = 0.026x + 0.001 and 0.9999 respectively. The recovery of the drug from the sample was ranged between 98.47% and 100.00%. The proposed method was validated as per ICH Q2 (R1) guidelines for precision, linearity, accuracy and recovery. The %RSD values for both intraday and interday precision were less than 2.0. The limit of detection (LOD) and limit of quantification (LOQ) were found to be 0.102µg/ml and 0.308µg/ml respectively by simple UV spectroscopy.

Key Words: Udenafil, UV-Spectroscopy, Validation, HCl, ICH guidelines

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-propoxy benzene-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.

MATERIALS AND METHODS

The spectrophotometric measurements were carried out using a Shimadzu UV-1700 UV/Vis spectrophotometer with 1cm matched quartz cell and Shimadzu ELB 300 analytical balance, Udenafil pure drug (99.99%) was obtained as a gift sample from Cadila Healthcare Ltd. All chemicals and reagents used were of analytical grade. Formulation used for studies was developed by Cadila Healthcare Ltd.

Preparation of Standard solution: Standard drug of Udenafil was proposed by dissolving 10mg pure Udenafil in 0.1N HCl and transferred into 10ml volumetric flask to obtain 1000µg/ml of stock solution. The standard solution of Udenafil having concentration of 20µg/ml was scanned in UV range

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(200-400nm) in 1.0 cm cell against in solvent as blank and spectrum was obtained.

Determination of λmax: 20µg/ml of Udenafil was prepared and scanned in UV range of 200-400nm and spectrum was obtained. The λmax was found to be at 291nm wavelength where absorbance was found maximum at this wavelength. Hence it is considered as absorbance maxima (λmax).

Preparation of calibration curve: Standard stock solution was suitably diluted with 0.1N HCl to obtain concentrations ranging from 5-35µg/ml. Absorbance of these solutions was measured at 291nm. Calibration curve was obtained by plotting graph between concentration and absorbance.

Preparation of test solution: 20 Tablets were weighed and its average weight was determined. An accurately weighed tablet powder equivalent to 10mg of Udenafil transferred into 100ml volumetric flask dissolved in 0.1N HCl, sonicated for 10min and volume was made up to the mark. Solution was filtered using whattman filter paper (No.41) and from that 2ml filtered solution was transferred into 10ml volumetric flask and made upto the mark with 0.1N HCl to obtain 20µg/ml solution.

Method Validation

Linearity: The absorbances were observed from 5 to 35µg/ml and were shown in Table-1. Linearity was obtained between 5 to 35µg/ml. Concentration graph was plotted for concentration and absorbance. The equation of calibration curve obtained was y = 0.026x + 0.001. The correlation coefficient (r) was found to be 0.9999.

Accuracy: To determine the accuracy of the method recovery was performed by standard addition method. To pre-analyzed sample known amount of standard Udenafil was spiked in different concentrations. The recovery was performed at three levels 50%, 100% and 150% of standard Udenafil. The solutions were prepared in triplicate and the accuracy was indicated by % Recovery.

Precision:

Repeatability: Six concentrations of 20µg/ml were prepared and the absorbances were read. The % RSD was calculated.

Intraday and Interday Precision: The concentration of 20µg/ml, 40µg/ml and 60µg/ml of Udenafil (on label claim basis) was taken. The absorbance of the final solution was read after 0hr, 12hr and 24hr in 1.0 cm cell at selected wavelength. Similarly the absorbance of the same solutions was read on 1st, 2nd and 3rd day. All the solutions are prepared triplicate and analyzed.

Ruggedness: It was carried out by analyzing the sample by two analysts and estimation of drug by proposed methods. The % RSD was calculated.

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 calculated.

RESULTS AND DISCUSSIONS

Attempt has been made to develop rapid, sensitive, economic, precise and accurate analytical method for Udenafil in pure and pharmaceutical dosage form. The proposed method is based on UV Spectrophotometric absorption in UV region using 0.1N HCl as solvent. Maximum absorbance was found to be at 291nm and is shown in **Figure: 1**. Beer's law was obeyed in concentrations ranging from 5 to 35µg/ml is incorporated in **Table: 1**. The correlation coefficient values were above 0.9999 which shows that absorbance was linear with concentration **(Figure: 2)**. The %Recovery studies were performed at 50%, 100% and 150% and was represented in **Table: 2.** Precision of the method was confirmed by Repeatability, Intraday and Interday analysis, %RSD values were represented in **Table: 3, 4 and 5**. LOD and LOQ were found to be 0.102µg/ml and 0.308µg/ml **(Table: 6)**. The ruggedness parameter was performed between two analysts and %RSD was found 0.19 and 0.11 **(Table: 7)**. The assay of the udenafil was performed and the results incorporated in **Table: 8.** The optical characteristics such as Beer's law limit, correlation coefficient, slope, intercept, molar absorptivity, scandell's sensitivity were calculated and validated **(Table: 9)**. The method was validated and found to be simple, sensitive, accurate and precise. Hence the proposed method could be effectively adopted for routine quality control of udenafil in bulk and formulated tablet dosage form.

CONCLUSION

The spectrophotometric method being reported for the assay of udenafil in pure form and also in its formulations is simple and inexpensive. The proposed method was found to be simple, sensitive, accurate and with good precision. Thus, this approach could be considered for the analysis of this drug in the quality control laboratories.

Acknowledgements: The authors are thankful to Cadila Healthcare Ltd for providing standard drug samples and also to Malla Reddy College of Pharmacy, for providing the facilities to carry out the work.

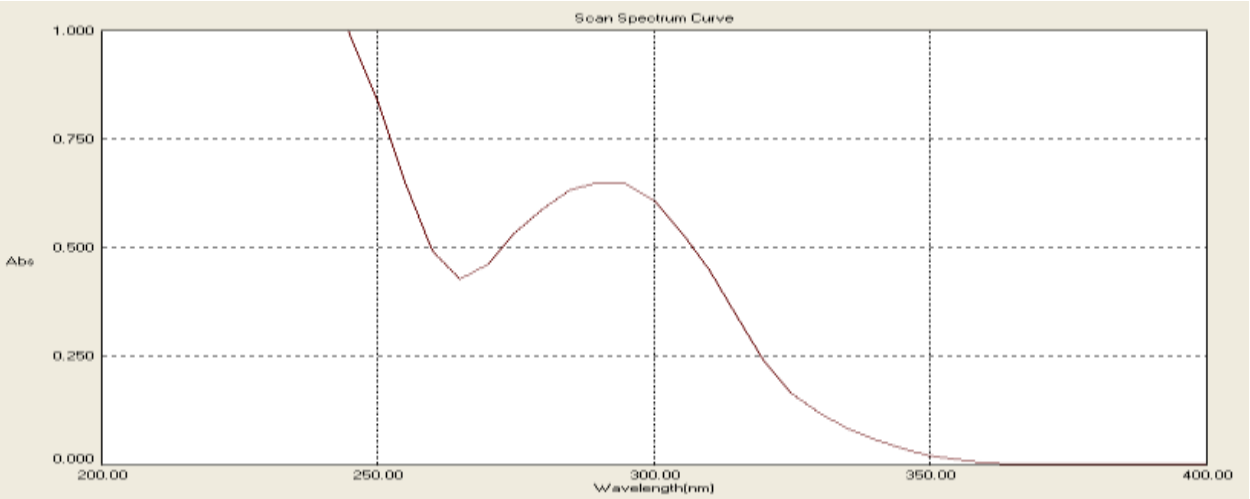

Fig - 1: UV Spectrum of Udenafil in 0.1N HCl at λmax = 291nm

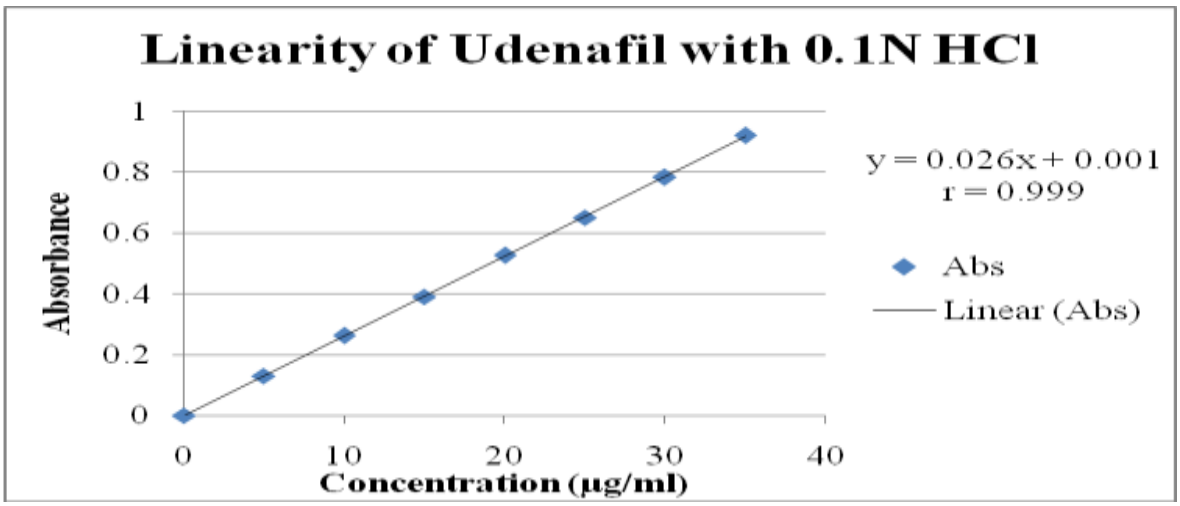

Fig - 2: Calibration curve of Udenafil in 0.1N HCl showing linearity relationship

Table 1: Calibration data for analysis of Udenafil in 0.1N HCl at λmax = 294nm

S.No.	Concentration (µg/ml)	Mean Absorbance (± SD)*
1	5	0.131 (± 0.0006)
2	10	0.263(± 0.0015)
3	15	0.391 (± 0.0006)
4	20	0.526 (± 0.0006)
5	25	0.651 (± 0.0006)
6	30	0.784 (± 0.0012)
7	35	0.921 (± 0.0006)

*n=3 (Average of 3 determinations)

Table-2: Recovery data of Udenafil in 0.1N HCl

Ingredient	Amount of drug from formulation	Amount of standard added	Percentage added	Amount added (µg/ml)	Amount found (µg/ml)	% Recovery (Mean ± RSD)*
Udenafil	10µg	5µg	50%	5	4.95	98.98 ± 0.45
Udenafil	10µg	10µg	100%	10	9.97	99.75 ± 0.22
Udenafil	10µg	15µg	150%	15	14.87	99.15 ± 0.15

*n=3 (Average of 3 determinations)

Table-3: Precision data of Udenafil in 0.1N HCl

S.No	Concentration(µg/ml)	Absorbance
1	20µg/ml	0.526
2	20µg/ml	0.526
3	20µg/ml	0.525
4	20µg/ml	0.526
5	20µg/ml	0.527
6	20µg/ml	0.525
Mean		0.526
Std dev		0.0008
%RSD		0.14

Table-4: Results of Intraday Precision of Udenafil in 0.1N HCl

Parameter	% Recovery Estimated (Mean ± RSD)*		
	10 (µg/ml)	20 (µg/ml)	30 (µg/ml)
At 0 hr	99.61 ± 0.38	99.86 ± 0.11	99.95 ± 0.07
At 12 hr	99.86 ± 0.22	99.74 ± 0.11	99.90 ± 0.15
At 24 hr	99.74 ± 0.44	99.80 ± 0.19	99.86 ± 0.13

*n=3 (Average of 3 determinations)

Table-5: Results of Inter-day Precision of Udenafil in 0.1N HCl

Parameter	% Recovery Estimated (Mean ± RSD)*		
	10 (µg/ml)	20 (µg/ml)	30 (µg/ml)
Day-1	101.12 ± 0.22	99.93 ± 0.11	100.03 ± 0.07
Day-2	99.86 ± 0.22	99.86 ± 0.22	99.95 ± 0.07
Day-3	99.99 ± 0.38	99.74 ± 0.22	99.99 ± 0.13

*n=3 (Average of 3 determinations)

Table-6: Lowest Limit of detection and Lowest Limit of quantification

LOD (µg/ml)	LOQ (µg/ml)
0.102	0.308

Table-7: Results of Ruggedness of Udenafil in 0.1N HCl

Ruggedness	%Purity ± RSD*
Analyst – 1	99.80 ± 0.19
Analyst – 2	99.74 ± 0.11

*n=3 (Average of 3 determinations)

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

Sample	Label	Amount found	% Purity ± RSD*
UDZIRE	100mg	99.94mg	99.93 ± 0.11

*n=3 (Average of 3 determinations)

Table-9: Validation Parameters

Parameters	Results
Absorption maxima λmax (nm)	291
Beer's law limit (µg/ml)	5-35
Molar Absorptivity (L mole^{-1}, cms^{-1})	1.3588 x 10^4
Sandell's sensitivity (µg/cm2/0.001)	0.0380
Correlation coefficient	0.9999
Regression equation	y = 0.026x + 0.001
Limit of detection	0.102
Limit of quantification	0.308
Precision(% RSD)	0.14

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