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# METHOD DEVELOPMENT AND VALIDATION OF UV-SPECTROSCOPIC METHOD FOR ESTIMATION OF UDENAFIL IN BULK AND TABLET FORMULATION

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**ABSTRACT**

Literature survey reveals that there are no analytical methods were reported for the estimation of Udenafil by UV method. Hence the present study aims in developing simple, rapid, precise and validated methods for Udenafil in bulk. The methods are Colorimetric determination of Udenafil by Visible Spectrophotometric method, Validation of Udenafil by Visible Spectrophotometric method. The suitable solvent selected for performing estimation of Udenafil by UV spectroscopic method development and validation and fixed the $\lambda_{max}$ for the drug Udenafil. The present study successfully estimated the Udenafil from the formulation and performed validation studies of the drug Udenafil.

**Key words:** Udenafil, UV spectroscopic method, validation studies.

## INTRODUCTION

Ultraviolet (UV) spectroscopy is a physical technique of the optical spectroscopy that uses light in the visible, ultraviolet, and near infrared ranges. The Beer-Lambert law states that the absorbance of a solution is directly proportional to the concentration of the absorbing species in the solution and the path length. Thus, for a fixed path length, UV/VIS spectroscopy can be used to determine the concentration of the absorber in a solution. It is necessary to know how rapidly the absorbance changes with concentration.

### Drug Profile

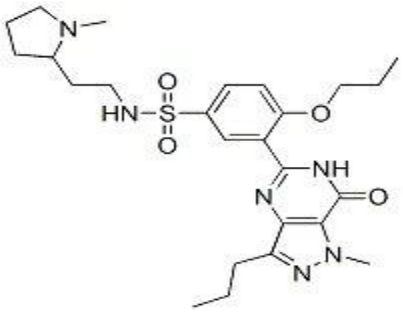
Udenafil is a new phosphodiesterase type 5 (PDE5) inhibitor used to treat erectile dysfunction (ED). It has been approved in South Korea and will be marketed under the brand name Zydena. It is not yet approved for use in the U.S., E.U., or Canada.

### Drug Monograph

Drug Profile Trade name: ZYDENA

Drug: Udenafil Indication: Treatment in erectile dysfunction and hypertension.
Categories: Vasodilator agents,phosphodiesterase inhibitors
Chemical Formula: $C_{25}H_{36}N_6O_{14}S$

**Figure 1. Chemical Structure of Udenafil**



**Indication:** Investigated for use/treatment in erectile dysfunction and hypertension.
**Pharmacodynamics:** Udenafil is a potent selective phosphodiesterase type 5 (PDE5) inhibitor.

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**Mechanism of action:** 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 C GMP [1-6].

## MATERIALS AND INSTRUMENTS

### Drug Sample

- The gift sample of pure drug Udenafil (Bulk powder) was received from MSN Labs, Hyderabad, India.
- The gift sample of Udenafil formulation (tablets) was received from MSN Labs, Hyderabad, India.

### Chemicals and solvents

- Methanol
- Distilled Water

### Instruments used

- Shimadzu UV Pharmspec Spectrophotometer 1700
- Shimadzu (ELB 300) Electronic balance
- Shimadzu (BL 22OH) Electronic balance

### Method A

### Preparation of the stock solution

A standard stock solution containing 1000µg/ml was prepared by dissolving 10 mg of Udenafil in 10ml Methanol. This stock solution used for further dilutions and by using analytical grade methanol as solvent for estimation.

### Preparation of the test solution

100 mg equivalent Udenafil formulation was taken and dissolved in 100ml of methanol by ultrasonication (for dissolving the excipients and insoluble residual particles). Then from this 10ml solution was taken and diluted to 100ml by using methanol. Then from this 1ml of solution was taken and diluted to 10ml in a 10ml standard flask. This gives concentration of methanol 10µg/ml. From this solution the concentration of 2,4,6,8 and 10µg/ml concentration solutions were prepared.

### Calibration curve preparation

Calibration curve or standard curve is a very important parameter for method validation and assay procedure for the substance. And this factor can be useful to estimate the assay value and drug content present in particular formulation of the drug. This calibration curve linearity can help to detect weather the proposed method is perfect or not. Calibration curve data were constructed in the range of the expected concentrations of 2µg/ml to 20µg/ml. *Beer's* law was obeyed over this concentration range. The regression equation was determined by using

$$y = mx + c.$$

Here C is concentration of analyte. By this calibration curve we can analyse the linearity of the method and range of the method.

**LOD** (**limit of detection)** LOD is defined as the lowest concentration of an analyte that an analytical process can reliably differentiate from back-ground levels. In this study LOD and LOQ were based on the standard deviation of response and the slope of corresponding curve using following equation:

$$LOD = 3.3\ \sigma / S$$

**LOQ (Limit of quantification)** LOQ is defined as the lowest concentration of calibration curve that can be measured with an acceptable accuracy, precision and variability. The value of LOQ was determined using following equation: These values are calculated by using the absorbance values of the sample in very low concentrations like 0.01µg/ml to 0.9µg/ml. from these values are done by randomly and identify the LOD and LOQ values accurately. Here "**σ"** indicates standard deviation of the absorbance and "**S"** indicates slope.

### Assay Procedure

Weigh accurately about 10 capsules powder and take 100mg equivalent quantity of udenafil and transfer into a 100ml standard flask. And dissolve the formulation in methanol by using ultrasonication. Then pipette out 10ml of solution and make up to 100ml leads to 10µg/ml concentration solution. This solution can be estimated in UV spectrophotometer by using methanol as blank at 293nm.

### Recovery Studies

Recovery studies were carried out by using spiking method. In this method the test sample having the concentration of 10µg/ml .To this standard drug is spiked by adding into the test solution. A concentration of 8, 10 and 12µg/ml are added to the sample solutions and the absorbances of the three spiked concentrations were taken. From this absorbance we can determine the amount of drug that can be recovered by the proposed method [7-12].

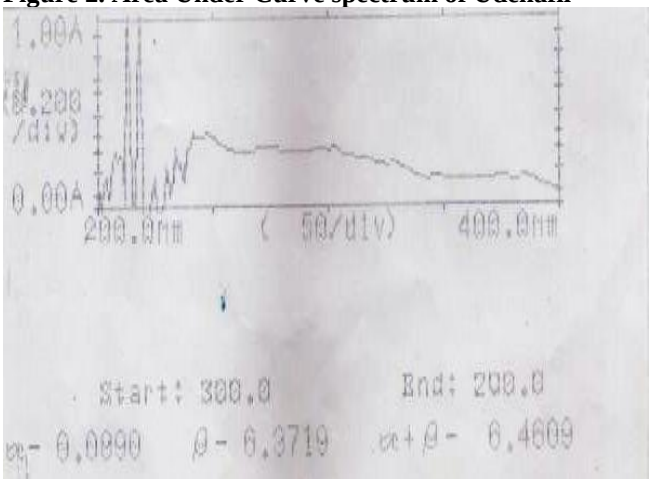
### Method B

### Area Under Curve Method

Area under curve method is applicable when there is no sharp peak and when broad spectra are obtained. Area calculation processing item calculation the area bound by the curve and horizontal axis. For the selection of analytical wavelength, 10 µg/ml solution of Udenafil was prepared by appropriate dilution of standard stock solution and scanned

in the range of 200-400 nm. From the spectra of drug, AUC in the range of 270-300 nm was selected for the analysis. The calibration curve was prepared in the concentration range of 4-20 µg/ml. By using the calibration curve, the concentration of the sample solution can be determined.

**Figure 2. Area Under Curve spectrum of Udenafil**

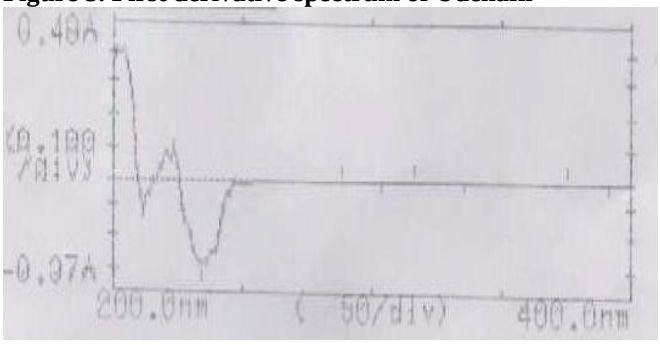



**Method C**

**First Derivative Spectroscopy**

In this method, 12 µg/ml solution of Udenafil was prepared by appropriate dilution of standard stock solution and scanned in the spectrum mode from 200-400 nm. The absorsption spectra thus obtained were derivatized for first order. First order derivative spectra of drug showed a sharp peak at 289 nm, which was selected for its quantification. The calibration curve for Udenafil was plotted in the concentration range of 2-20 µg/ml at wavelength 289 nm. The concentration of drug in test solution was determined against the calibration curve in quantitation mode [13-16].

**Figure 3. First derivative spectrum of Udenafil**




## RESULTS AND DISCUSSION

**Method Development Parameters**

**Selection of Solvent**

Udenafil is a highly lipophilic drug that is practically insoluble in water, acid solutions, alkaline solutions and phosphate buffer solutions. But it shows good solubility in methanol and ethanol. Among these, Methanol was found to be most suitable solvent for UV spectroscopic method than ethanol because of the linearity and reproducibility of the value.

**Preparation of stock Solutions**

A standard stock solution containing 1000µg/ml was prepared by dissolving 10 mg of Udenafil in 10ml Methanol. This stock solution used for further dilutions and by using analytical grade methanol as solvent for estimation.

**Fixing of wave length**

After selecting the suitable solvent, the fixing of the λmax for the proposed method is very important. This can be done by scanning the drug sample (Udenafil) solution in Methanol in the range of 400nm-200nm and the most repeated maximum absorbance with linearity and repeatability can be fixed as λmax for the drug. And in the proposed method for Udenafil drug shows maximum 293 nm, with more linearity, repeatability (ruggedness) and the λmax was fixed as 293 nm shown in the (figure 4).

**Figure 4. U.V. spectrum of Udenafil drug (200-400nm)**

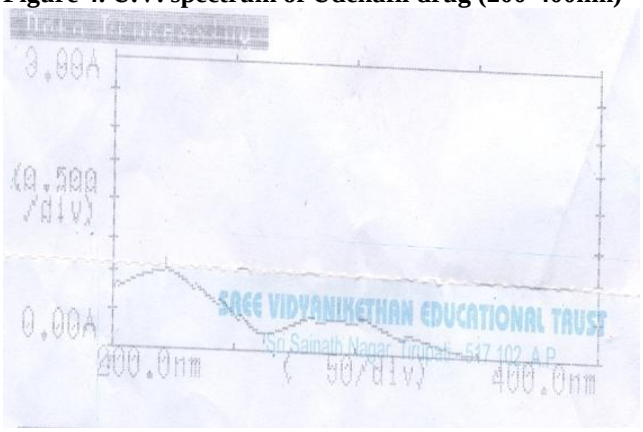



**Table 1. Standard curve values**

| Concentration(µg/mL) | Absorbance |
|---|---|
| 2 | 0.072 |
| 4 | 0.140 |
| 6 | 0.220 |
| 8 | 0.290 |
| 10 | 0.358 |
| 12 | 0.450 |
| 14 | 0.529 |
| 16 | 0.600 |
| 18 | 0.670 |
| 20 | 0.715 |

**Calibration curve preparation**

Calibration curve or standard curve is a very important parameter for method validation and assay procedure for the substance. And this factor can be useful to estimate the assay value and drug content present in

particular formulation of the drug. This calibration curve linearity can help to detect weather the proposed method is perfect or not.

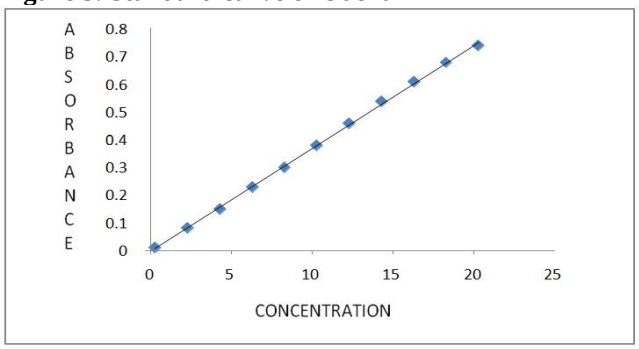
Calibration curve data were constructed in the range of the expected concentrations of 2µg/ml to 20µg/ml *Beer's* law was obeyed over this concentration range. The regression equation was found to be

**y = 0.036x + 0.054.**

The correlation coefficient (r) of the standard curve was found to be 0.9968.The stock solutions and working standards were made in methanol.

Linearity range and calibration curve is presented in Table 1 and Figure 4.

**Figure 5. Standard curve of Udenafil**




**Beer's limit**

The limits in which beers law obeyed is beers limit. In the UV method, accuracy, precision, ruggedness and robustness should be within Beer's limit. And for Udenafil the beers limit range was found to be 2µg/ml to 20µg/ml. Within this range the drug showed accuracy, linearity, precision, ruggedness, robustness.

**Molar Absorptivity**

This is the important factor for determining the absorptive property of a drug in 1 mole concentration. And this value can be useful in determining the absorbance of drug in molar concentrations. This for identifying the shifts of the maximum absorbance of the drug during the method development. The molar absorptive of the Udenafil was found to be 3.94 $x10^4$L $mole^{-1}$ $cm^{-1}$.

**Linearity**

The linearity of the assay was determined by plotting standard calibration curves for the concentration range 2-20 µg/ml at 293 nm using methanol as solvent. The methods for estimation of Udenafil in methanol was found to be linear in the range of concentrations 2-20 µg/ml as suggested by the linear least square regressions (>0.999) of the standard curves.

**Precision and accuracy**

Precision is degree of repeatability of an analytical method under normal operation conditions. The precision and accuracy were determined with standard quality control samples prepared in triplicate at different concentration levels covering the entire linearity range. The precision of assay was determined by repeatability (intraday), intermediate precision (inter day) and reported as % RSD for a statistically significant number of replicate measurements. The intermediate precision was studied by comparing the assay on three different days and the results are documented as the standard deviation and % RSD. Accuracy is the percentage of analyte recovered by assay from known added amount. Data from nine determinations over three concentration levels covering the specified range were obtained.

**LOD (Limit of Detection)**

LOD is defined as the lowest concentration of an analyte that an analytical processcan reliably differentiate from back-ground levels. In this study LOD and LOQ were based on the standard deviation of response and the slope of corresponding curve. The LOD was found with in limit concentrations that 0.113 µg.

**Method Validation Parameters of Udenafil**

**Table 2. Characteristics of Udenafil**

| Parameters | Method-A | Method-B | Method-C |
|---|---|---|---|
| $\lambda$max | 293 nm | 270-300 nm | 275 nm |
| Beer's law limit | 2-20 µg/ml | 4-20 µg/ml | 2-10µg/ml |
| Molar absorptivity | 3.094 $x10^4$L $mole^{-1}$ $cm^{-1}$ | 3.094 $x10^4$L $mole^{-1}$ $cm^{-1}$ | 3.094 $x10^4$L $mole^{-1}$ $cm^{-1}$ |
| Regression equation (Y =mx + c) | y = 0.03909x +0.0014 | | |
| Slope (m) | 0.036 | 0.398 | 0.032 |
| Intercept (c) | 0.054 | 0.0034 | 0.0036 |
| Correlation coefficient ($r^2$) | 0.999 | 0.998 | 0.988 |
| Relative standard deviation(%) | 0.237 | 0.352 | 0.472 |
| LOD Value | 0.11 | 0.11 | 0.11 |
| LOQ Value | 0.354 | 0.35 | 0.35 |

**Table 3. Assay value of Udenafil**

| Drug | Label claim mg/tab | Amount found | %Purity |
|---|---|---|---|
| Udenafil | 150 | 9.95 ±0.21 | 92.80 %w/w |

**Table 4. Udenafil intra-day and inter-day precision**

| Sample (µg/ml) | Intra-day precision | %RSD | Inter-day precision | %RSD |
|---|---|---|---|---|
| 8 | 0.290<br>0.296<br>0.299 | 1.532 | - | - |
| 12 | - | - | 0.450<br>0.458<br>0.464 | 1.74 |

**Table 5. Recovery studies (accuracy parameter) of Udenafil**

| Test µg/ml | Level % | Amount of standard drug added µg/ml | %Recovery | Standard deviation |
|---|---|---|---|---|
| 10 | 80 | 8 | 99.6 | 0.161 |
| | 100 | 10 | 99.8 | 0.181 |
| | 120 | 12 | 101.0 | 0.078 |

**Table 6. Area Under Curve Studies of Udenafil**

| Concentration(µg/ml) | AUC values |
|---|---|
| 2 | 1.0176 |
| 4 | 1.3669 |
| 6 | 2.4548 |
| 8 | 3.4346 |
| 10 | 3.7543 |
| 12 | 4.3660 |
| 14 | 5.4424 |
| 16 | 7.4902 |
| 18 | 6.9172 |
| 20 | 7.5769 |
| Slope | 0.398 |

**Figure 6. AUC Calibration Graph of Udenafil**

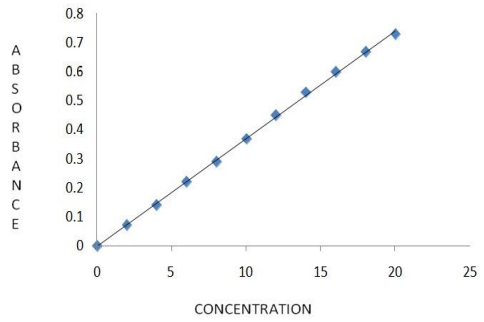
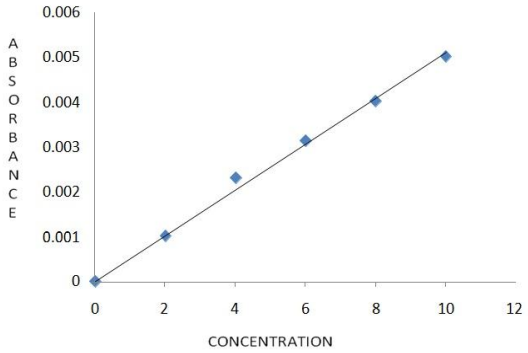



**Table 7. 1st Derivative Studies of Udenafil**

| Concentration(µg/ml) | Absorbances |
|---|---|
| 2 | 0.002 |
| 4 | 0.003 |
| 6 | 0.005 |
| 8 | 0.007 |
| 10 | 0.008 |
| Slope | 0.032 |

**Figure 7. First Derivative calibration graph of Udenafil**




**LOQ (Limit of Quantification)**

LOQ is defined as the lowest concentration of calibration curve that can be measured with an acceptable accuracy, precision and variability. LOQ (Limit of Quantification) Value is the minimum quantity of drug that can be quantified by the instrument and the value was found to be 0.354 µg/ml.

**Assay Procedure**

Weigh accurately about 10 tablets and take 100mg equivalent quantity of Udenafil and transfer into a 100ml standard flask. And dissolve the formulation in methanol by using ultrasonication. Then pipette out 10ml of solution and make up to 100ml leads to 10µg/ml concentration solution. This solution can be estimated in UV spectrophotometer by using methanol as blank at 293nm. The percentage purity of the drug was found to be 99.53% w/w and amount drug was found to be around 78.87 ± 0.218 mg/ tablet (Table 2).

**Procedure for the Estimation of Udenafil in Formulation**

A commercial formulation of Udenafil (200 mg) tablet was obtained as gift sample from MSN Labs, Hyderabad, India. The content of 20 tablets were crushed and accurately weighed amount of the contents equivalent to 100 mg of Udenafil was transferred into 100 ml volumetric flask and make up the volume with methanol and sonicate it for a while until the drug completely dissolved. The content of the flask was filtered through whatmann filter paper No.1 and 10 ml of the filtrate was diluted up to 1000 ml with methanol. And this solution is makeup to 10 ml and performs a series of dilutions from 2µg/ml to 25µg/ml and subjected to UV spectroscopy. A standard curve was prepared based on the measured absorbance values by keeping λmax of the drug as 293 nm.

**Analytical method validation**

The data was statistically validated by means of least square regression method. The standard solution placed in room temperature in methanol solution and degradation was not observed. Repeatability of values was observed over a period of 48 hours. The data was statistically validated by means of least square regression method. The detection and quantification limits were found to be 0.19 and 0.634. The mean percentage drug estimated was 100.37 indicating the accuracy of the proposed analytical method (Table 3).

Mean standard deviation for Udenafil at 80, 100 and 120 % was found to be 0.161, 0.181 and 0.078 respectively. The low values of these statistical parameters validated the method. The value of mean percentage recovery was 99.6, 99.8 and 101.0 (Table 5). These facts, together with satisfactory low values of statistical parameters, further validated the method. There was no interference of excipients in the estimation. The proposed method can be successfully employed in the routine analysis of Udenafil containing dosage forms.

**DISCUSSION AND CONCLUSION**

This current investigation is intended to develop three methods to estimate the Udenafil by U.V. Spectrophotometry and validate the methods, for both bulk and formulations of Udenafil. Three methods for the determination of Udenafil in the bulk drug and formulation have been developed. In Method-A from the spectrum of Udenafil, it was found that the maximum absorbance is at 293 nm in methanol. A good linear relationship (0.999147) was observed between the concentration ranges of 2-20 µg/ml. The assay of tablet was found to be 99.53%. The high percentage recovery indicates the high accuracy of the method. Method-B (Area Under Curve method) obeyed Beer Lamberts law in the concentration range 4-20 µg/ml. Method-C (First order derivative spectra) the range is 2-20 µg/ml.

These methods involves direct analysis without any extraction steps, thus it is performed faster, simple and easier. And this methods showed accurate and précised results. By these results these methods were found to be rapid, simple, accurate, and economic for analysis and quality determination. Thus the three developed methods can be easily applied for the routine quality control of Udenafil in bulk and tablet dosage form.

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