RESEARCH ARTICLE Am. J. PharmTech Res. 2013; 3(2) ISSN: 2249-3387

AMERICAN JOURNAL OF PHARMTECH RESEARCH

**Journal home page:** http://www.ajptr.com/

# Determination of sildenafil citrate and its related substances in the commercial products and tablet dosage form using RP-UPLC.

**Srihari Molleti*[1], Vinay Rao[2], K. N. Jayaveera[3]**
*1. Daewoong Pharmaceuticals India Private limited, Balanagar, Hyderabad India.*
*2. Malla reddy college of pharmacy, Hyderabad*
*3. Department of chemistry, JNT University, Anantapur. India.*

## ABSTRACT

This study is aimed at Developing and validating an UPLC method for the related substances of Sildenafil that might coexist in tablet formulations as impurities that may originate from synthesis process or degradation. A chromatographic system consisting Waters Acquity UPLC HSS C18(1.8 µm)column, mobile phase of ammonium acetate and Acetonitrile with gradient elution at flow of 0.3 mL/min and UV detector set at 245 nm has shown a good chromatographic separation for Sildenafil and its related substances. The developed method was validated as per ICH Guidelines and compared with Pharma Europa method. The pharmacopeia method has above 60 minutes runtime to separate all the listed related compounds, the developed UPLC method has run time of only 10 minutes making the method productive and may be applied for Quality control Testing.

**Key words:** Sildenafil citrate, Stability indicating, RP-UPLC.

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*Corresponding Author Email: m.hyderabad@gmail.com
Received 10 February 2013, Accepted 19 February 2013

Please cite this article in press as: Molleti S., *et al.*, Determination of Sildenafil citrate and its related substances in the commercial products and tablet dosage form using RP-UPLC. American Journal of PharmTech Research 2013.

# INTRODUCTION

Sildenafil citrate[1] is a therapy used for erectile dysfunction by acting as a selective inhibitor of cyclic guanosine mono phosphate(cGMP)-specific phosphor diesterase type 5(PDES).Sildenafil is chemically known as 1-[[3-(6,7-dihydro-1-methyl-7-oxo-3-propyl-1 H pyrazolo[4,3-d]pyrimidine-5-yl)-4-ethoxy phenyl]sulfonyl]-4-methyl piperizine citrate has the following structural formulae$C_{28}H_{38}N_6O_{11}S$. And its molecular mass: base: 474.6 g mol−1; citrate: 666.7 g mol−1. Sildenafil enhances relaxation of the corpus cavernosal smooth muscle, which in turn increases blood flow into the cavernosal spaces, thus leading to increased intra cavernosal pressure, a key factor in producing an erect penis[2-3].

Sildenafil citrate, sold under the names Viagra, was a drug used to treat male erectile dysfunction (impotence) and pulmonary arterial hypertension (PAH). 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. Pistos et al[4] have proposed a HPLC method for determination of sildenafil and its active metabolite (N-desmethyl sildenafil) in human blood. Determination of sildenafil citrate in human plasma [5-9] and in pharmaceutical formulations[10-15] using chromatographic methods has been reported.

Ultra performance liquid chromatography (UPLC) is a new category of separation science which builds upon well established principles of liquid chromatography, using sub 2 µm porous particles. These particles operate at elevated mobile phase velocities to produce rapid separations with increased sensitivity and increased resolution. Thus UPLC technology allows analysts time to be drastically reduced while still meeting assay acceptance criteria based on plate count, resolution and analyze retention.

Extensive literature survey revealed that, Ultra performance liquid chromatography method was not reported for the sildenafil and its related substances in its Bulk drugs and its formulations. Reports have been appeared describing accurate analytical and spectrophotometric techniques for quantification and stability assay of sildenafil. For related substances [16-38] was reported most of these methods are time consuming, expensive, suffer from lack of selectivity and require careful control of conditions and considerable time for routine control analysis.

The HPLC method of analysis as reported in the pharmacopoeia is adequate to separate all the process related as well as the probable degradation related substances in both the API as well as finished dosage form. However, this method suffers from an inordinately long run time (60>)

and hence is inherently expensive. The current paper describes the development and validation of a UPLC method which separates all the monograph listed impurities within a short span of 10 minutes run time. The method equivalency with the Pharmacopeia method was established.

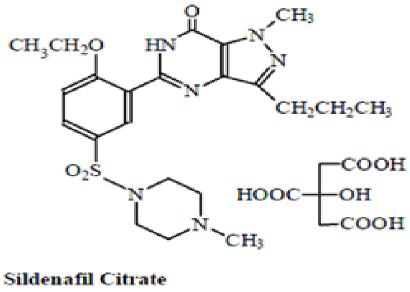

**Figure1: Sildenafil citrate chemical structure**

## MATERIALS AND METHODS:

### Chemicals:

All the chemicals used were of pharmaceutical grade. Acetonitrile of chromatographic grade and extra pure ammonium acetate were from Merck. Sildenafil citrate and its impurities were obtained from Hetero drugs limited. Viagra tablets were obtained from Pfizer.

The compounds related to sildenafil which could be expected as impurities or might appear as degradation products have been prepared and identified and listed in Pharma Europa 2.2.29. Related compound-01: 4-(2-Ethoxy benzamido)-1-methyl-3n-propyl-pyrazole-5-carboxamide. Related compound-02:5-(2-ethoxy phenyl)-1-methyl-3n-propyl-1,6-dihydro-7H-pyrazolo[4,3-d]pyrimidine-7-one. Related compound-03:1-[[3-4, 7-dihydro-1-methyl-7-oxo-3-propyl-1H-pyrazolo [4, 3-d] pyrimidine-5-yl)-4-ethoxy phenyl] sulfonyl]-piperizine. Related compound-04:5-{2-ethoxy-5-[(4-{[4-ethoxy-3-(1-methyl-7-oxo-3-propyl-6,7-dihydro-1H-pyrazolo[4,3-d]pyrimidin-5-yl)phenyl]sulfonyl}piperizino)sulfonyl]phenyl}-1-methyl-3-propyl-6,7-dihydro-1H-pyrazolo[4,3-d]pyrimidin-7-one.

### Developing an UPLC Method:

The UPLC method carried out in this study aimed at developing a chromatographic system capable of eluting and resolving sildenafil and its impurities (related substances) from one another and that complies with the general requirements for system suitability.

The preliminary investigations were directed to-wards the effect of various variables on the system suitability of the method. The parameters assessed include the detection wavelength, the type and quantity of the organic modifier, the column, the salt concentration and the pH of the mobile phase.

Sildenafil citrate showed two UV-absorption maxima at 220 –295 nm. The 245-nm wavelength showed a better resolution between the chromatographic peaks of sildenafil and its impurities and the absorption measurements at this wavelength were of optimal values for sildenafil citrate and other related compounds.

The first trial was carried out by using reversed phase C18column (BEH,C-18,2.1x100mm) and a mixture of acetonitrile-0.1 M ammonium acetate (7:3, v/v).This system was found to be suitable to elute sildenafil but the retention time was short and the column resolution towards sildenafil and its impurities was low. As a means to increase the retention time, the Initial percentage of acetonitrile portion in the mobile phase was decreased from 70 to 45%.

The results showed that the retention time was increased but the tailing factor was so high for the sildenafil peak. However, the increase of the modifier ratio was associated by a decrease in the retention times of all the compounds. Replacing the acetonitrile organic modifier, with methanol was associated by a high tailing factor and longer retention times. When the Acquity C18 column of 5mm was then replaced by HSS C18 column of 100mm, the tailing factor was reduced with better peak characteristics. Changing the concentration of ammonium acetate from 0.1 to 0.2 M had resulted in an enhancement to the peak symmetry and a reduction in the tailing factor. When the pH of the mobile phase was decreased from 7 to 5, the chromatographic peaks showed a decrease in the retention times and the column resolution for the impurities became low.

Consequently, the optimum chromatographic conditions mentioned previously were applied for all measurements. Figure. 2 shows the significant separation of sildenafil and its related compounds using the optimised chromatographic system.

**Finalized conditions:**

Gradient elution technique was utilized with the column maintaining at 45°C, The Buffer phase used was ammonium acetate0.2M (15.416 grams per 1000mL) and organic phase was acetonitrile with the following gradient programme. The flow rate was 0.3 mL/min. Samples of 1µL was injected into the column and the detector was set at 245 nm with run time of 10 minutes. The relative standard deviation (R.S.D.) of six replicate injections of the standard preparation was not greater than 5.0% and the tailing factor was less than 2.0.

**Table 1: Gradient programme**

| Time(min) | Flow(mL/min) | %A | %B |
|---|---|---|---|
| 0.00 | 0.3 | 55 | 45 |
| 4.00 | 0.3 | 40 | 60 |
| 6.00 | 0.3 | 15 | 85 |
| 8.00 | 0.3 | 55 | 45 |
| 10.00 | 0.3 | 55 | 45 |

## PREPARATION OF SOLUTIONS:

### Preparation of diluted standard solution

An accurately weighed quantity of sildenafil citrate and its related substances (Impurities A, B, C and D) was dissolved in the mobile phase and diluted quantitatively. Serial dilutions were carried out, using the mobile phase, to obtain solutions of known concentrations to be used for the standard reparation 100 ppm for assay and 5 ppm for Sildenafil diluted standard.

### Preparation of Test solutions (In-House):

Equivalent weight of 100 mg sildenafil citrate accurately weighed and disintegrated with the 20 mL of water and diluted quantitatively to 100 ml using Acetonitrile. The solutions were sonicated for 30 min, centrifuged at 4000 rpm for 10 min and the supernatant was used to as a test solution for related substances test, the filtered solution diluting 5 mL to 50 mL used for Assay.

### Preparation of Test solutions (Viagra):

Fifty tablets were powdered and accurately weighed portions equivalent to 100 mg sildenafil Citrate were transferred to100 ml volumetric flasks. Disintegrated with 20 mL of water and Acetonitrile was added and the solutions were sonicated and centrifuged as above and the supernatant was used as Related substances test solution with 1.0 mg sildenafil citrate per ml, the filtered solution diluting 5 mL to 50 mL used for Assay.

### Quantification:

Equal volumes, (1µL), of the standard preparations and the test preparations that contain Sildenafil citrate related substances and its related substances were injected into the chromatograph and the chromatograms were recorded. The responses (peak area) for the major peaks were measured and the quantity of sildenafil or related substance was calculated from the equation Cs (Au / As) where Au and As are the areas under the corresponding peaks and Cs is the concentration of sildenafil or related substance in the standard solution.

## METHOD VALIDATION

The method validation was performed as per ICH Guidelines[39].

### Linearity, Limit of Detection, Limit of Quantification

The degree of linearity was assessed by the correlation coefficient, y-intercept, and slope. The, LOD and LOQ have been estimated for related substances as 3 S.D. and 10 S.D. of the y intercept and slope.

### Precision

The precision was performed by preparing six individual preparations as per the method of analysis and evaluated for percentage of Sildenafil and its percentage of individual and total impurities.

**Accuracy**

The samples were prepared by spiking the Sildinafil and its impurities stock solutions into the Placebo mixture and the percent recovery was estimated.

**Solution stability**

The solutions prepared was tested at initial, 24hrs and 48Hrs by maintaining at room temperature and estimated for Sildenafil and its impurity content.

**Robustness**

Robustness was conducted by making the variations in flow rate, Column oven temperature and percentage of Acetonitrile.

**Ruggedness**

The prepared solutions were filtered through 0.45 µ PVDF syringe filter and 0.45 µ PVDF syringe filter and evaluated against the centrifuged sample.

**Intermediate precision**

The test was performed with another analyst on different day, different system and different column and the impurity contents were reported.

**Forced degradation studies**

The forced degradation studies conditions and % degradation s mentioned in the results (Table: 6) section.

**Study for Uneluted peaks:**

Since the runtimes are lower, a study conducted on all the stressed samples for knowing the retained peaks by increasing the acetonitrile to 90% for 20 minutes.

**Equivalency with the Pharmacopeia method:**

The equivalency with pharmacopeia method was established in three steps.

**System suitability equivalence:**

The System suitability parameters in the pharmacopeia method and developed method were compared with the obtained values.

**API Analysis equivalence:**

The results obtained with the Same API batch analysis with the pharmacopeia method and the developed method, results were discussed.

**Reference Product analysis equivalence:**

The results obtained with the Same Viagra tablet batch analysis with the pharmacopeia method and the developed method, results were discussed.

## RESULTS AND DISCUSSIONS

After achieving the final conditions the impurity mixture chromatogram was as shown below.

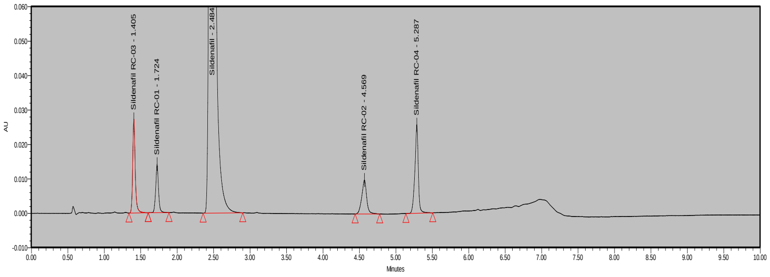

**Figure 2: Impurities spiked sample chromatogram.**

### Assay method validation results:

### Specificity:

### Blank interference:

The diluent was injected as a blank; it was found that there was no interference observed in blank preparation with the Sildenafil peak.

### Placebo interference:

With the equivalent weight of sample the placebo preparation was prepared and injected into the system and interference checked, it was found that there was no interference observed in placebo preparation with the Sildenafil peak.

### Impurity interference:

Impurity solution was prepared at 5 % level of test concentration and injected into the system and checked for the interference, it was found that impurities are not interfering with the sildenafil peak.

### Linearity:

The linearity was performed at 6 levels of the targeted 100 ppm (25%, 50%, 80%, 100%, 200%, 300% levels) and the area results are plotted against the concentration, the correlation coefficient observed was 0.9998.

### Precision:

By following the procedure in six sample preparations were prepared and calculated the assay values and the Percent relative standard deviation was 0.4 shows that the method was precise as per the ICH limits.

### Accuracy:

The accuracy was performed in triplicate by spiking the sildinafil citrate into the placebo mixture at 50%, 100%, and 200% 300% of test concentration; from the area recovery values are calculated. The average recovery values are obtained within 98 to 102% shows that the method was accurate as per the ICH limits.

**Solution stability:**

The first three solutions prepared in the 3.1.3 section were checked for sildenafil content at 24 and 48 hours by keeping it in closed container at room temperature, the variation from the initial value was 0.11% at 24 Hours and 0.13% at 48 hours, the results are within 1% shows the solution was stable for 48 hours.

**Robustness:**

To check the effect of deliberate changes in the method, the variation inflow rate (±0.05 mL) and variation in temperature (± 5ºC) are studied; result shows no effect on the method.

**Rugged ness:**

From the stock solutions of 3.1.3 the solution are prepared by filtering through PVDF & PTFE 0.45 µm filter papers and the content of sildinafil was tested. The results obtained are within 1%.

**Intermediate precision:**

Assay was performed by another analyst on different day, different system, and different column; the variation between the two analysts was less that 0.1 %, it shows that method was reproducible.

**Table 2: Validation results of Sildenafil citrate in assay method**

| Parameter | Results | |
|---|---|---|
| Specificity | Blank interference, Placebo interference, Impurity interference was nil. | |
| Linearity | Established from 25% to 300% (R2 value=0.9998) | |
| Precision: | % RSD of impurity for six preparations= 0.4 | |
| Accuracy | %Level | %Recovery |
| | 50% | 99.1 |
| | 100% | 99.9 |
| | 200% | 100.2 |
| | 300% | 100.5 |
| Solution stability | 1)% Difference at 24 Hrs=0.11%<br>2)%Difference at 48 Hrs=0.13% | |
| Robustness | Flow rate variation-System suitability passes Temperature variation system suitability passes | |
| Ruggedness | Filter validation: Variation between PVDF &PTFE 0.45 micron filters=0.21% | |
| Intermediate precision | % Assay variation from the precision sample.03% | |

**Impurity method validation results:**

**Specificity:**

**Blank interference:**

The diluent was injected as a blank; it was found that there was no interference observed in blank preparation with the Impurity peaks.

**Placebo interference:**

With the equivalent weight of sample the placebo preparation was prepared and injected into the system and interference checked, it was found that there was no interference observed in placebo preparation with the Impurity peaks.

**Impurity interference:**

Impurity solution was prepared at 5 % level with the sildinafil API at test concentration and injected into the system and checked for the interference, it was found all the impurities are separated with minimum resolution of 3.0, indicating no impurity interference.

**Linearity:**

The linearity was performed at 8 levels of the targeted diluted standard concentration 5 ppm (10%, 20%, 50%, 80%, 100%, 120%, 150% and 300% levels) and the area results are plotted against the concentration, the correlation coefficient observed was above 0.998 shows that the method was linear.

**Precision:**

By following the procedure in 2.4.2 section, six sample preparations are prepared and calculated the impurity content values and the Percent relative standard deviation for four impurities was below 1% shows that the method was precise.

**Accuracy:**

The accuracy was performed in triplicate by spiking the impurity stock solutions into the placebo mixture at 10%, 20%, 50%, 80% and 100% 120% of diluted standard concentration; from the obtained area recovery values are calculated. The average recovery values are obtained within 85 to 115% shows that the method was accurate as per the ICH limits.

**Solution stability:**

The first three solutions prepared in the 3.2.3 section were checked for individual and total impurities contents at 24 and 48 hours by keeping it in closed container at room temperature, the variation from the initial individual impurity content and total impurity content value was below 0.05% shows the solution was stable for 48 hours.

**Robustness:**

To check the effect of deliberate changes in the method, the variation inflow rate (±0.05 mL) and variation in temperature (± 5ºC) are studied; result shows no effect on system suitability and resolution 3.0 was maintained in all the changes.

**Ruggedness:**

From the stock solutions of 3.2.3 the solution are prepared by filtering through PVDF & PTFE 0.45 µm filter papers and the content of individual impurities and total impurities content for both filters was tested. The results obtained are within 0.05%.

**Intermediate precision:**

Impurity test was performed by another analyst on different day, different system, and different column; the variation between the two analysts was less that 0.1 %, it shows that method was reproducible. The validation results obtained with the related compounds are summarized in below (Table 3, 4, 5, 6)

**Table 3: Validation results of related compound-1 in impurities method**

| Parameter | Results | |
|---|---|---|
| Response factor | 1.39 | |
| Specificity | Blank interference, Placebo interference, Impurity interference was nil. | |
| Linearity | Established from 0.43 ppm to 16.35 ppm (R2 value=0.9998) | |
| LOD and LOQ | LOD=0.009 ppm and LOQ=0.03 ppm | |
| Precision: | % RSD of impurity for six preparations= 1.61 | |
| Accuracy | %Level | %Recovery |
| | 10% | 102.4 |
| | 20% | 93.4 |
| | 50% | 92.1 |
| | 80% | 105.1 |
| | 100% | 111.4 |
| | 120% | 111.4 |
| Solution stability | 1)% Difference at 24 Hrs=0.01%<br>2)%Difference at 48 Hrs=0.01% | |
| Robustness | Flow rate variation-System suitability passes<br>Temperature variation system suitability passes | |
| Ruggedness | Filter validation:<br>Variation between PVDF &PTFE 0.45 micron filters=0.01% | |
| Intermediate precision | Individual impurity variation=0.03%<br>Total impurity variation=0.03% | |

**Table 4: Validation results of related compound-2 in impurities method**

| Parameter | Results | |
|---|---|---|
| Response factor | 0.83 | |
| Specificity | Blank interference, Placebo interference, Impurity interference was nil. | |
| Linearity | Established from 0.49 ppm to 18.60 ppm (R2 value=0.9999) | |
| LOD and LOQ | LOD=0.12 ppm and LOQ=0.37 ppm | |
| Precision: | % RSD of impurity for six preparations= 0.48 | |
| Accuracy | %Level | %Recovery |
| | 10% | 94.5 |
| | 20% | 89.6 |

| | | |
|---|---|---|
| | 50% | 88.2 |
| | 80% | 104.0 |
| | 100% | 110.3 |
| | 120% | 109.4 |
| Solution stability | 1)% Difference at 24 Hrs=0.01%<br>2)%Difference at 48 Hrs=0.01% | |
| Robustness | Flow rate variation-System suitability passes<br>Temperature variation system suitability passes | |
| Ruggedness | Filter validation:<br>Variation between PVDF &PTFE 0.45 micron filters=0.00% | |
| Intermediate precision | Individual impurity variation=0.03%<br>Total impurity variation=0.03% | |

**Table 5: Validation results of related compound-3 in impurities method**

| | | |
|---|---|---|
| Response factor | 0.91 | |
| Specificity | Blank interference, Placebo interference, Impurity interference was nil. | |
| Linearity | Established from 0.76 ppm to 28.65 ppm (R2 value=1.0) | |
| LOD and LOQ | LOD=0.23 ppm and LOQ=0.69 ppm | |
| Precision: | % RSD of impurity for six preparations= 0.54 | |
| Accuracy | %Level | %Recovery |
| | 10% | 105.5 |
| | 20% | 96.2 |
| | 50% | 91.5 |
| | 80% | 105.3 |
| | 100% | 101.8 |
| | 120% | 101.4 |
| Solution stability | 1)% Difference at 24 Hrs=0.01%<br>2)%Difference at 48 Hrs=0.01% | |
| Robustness | Flow rate variation-System suitability passes<br>Temperature variation system suitability passes | |
| Ruggedness | Filter validation:<br>Variation between PVDF &PTFE 0.45 micron filters=0.00% | |
| Intermediate precision | Individual impurity variation=0.01%<br>Total impurity variation=0.03% | |

**Table 6: Validation results of related compound-4 in impurities method**

| | | |
|---|---|---|
| Response factor | 0.83 | |
| Specificity | Blank interference, Placebo interference, Impurity interference was nil. | |
| Linearity | Established from 0.62 ppm to 23.55 ppm (R2 value=0.9998) | |
| LOD and LOQ | LOD=0.2 ppm and LOQ=0.61 ppm | |
| Precision: | % RSD of impurity for six preparations= 0.54 | |
| Accuracy | %Level | %Recovery |
| | 10% | 94.5 |
| | 20% | 87.6 |
| | 50% | 87.1 |
| | 80% | 104.4 |
| | 100% | 112.2 |
| | 120% | 110.4 |
| Solution | 1)% Difference at 24 Hrs=0.04% | |

| | |
|---|---|
| stability | 2)%Difference at 48 Hrs=0.03% |
| Robustness | Flow rate variation-System suitability passes<br>Temperature variation system suitability passes |
| Ruggedness | Filter validation:<br>Variation between PVDF &PTFE 0.45 micron filters=0.01% |
| Intermediate precision | Individual impurity variation=0.04%<br>Total impurity variation=0.05% |

**Study of uneluted peak:**

The study shows that no peak eluted, proved that there was no unretained peak with the developed method.

**Forced degradation studies:**

To prove the stability indicating power of the method the forced degradation studies are carried out. The degradation reagents were (30 mL) added after the disintegration kept on reflux for the specified time. In each condition the individual % of impurities and total impurities and assay are calculated. The mass balance obtained from the experiment was ranged 99.5 to 100.0%. In all the forced degradation conditions peak purity of sildinafil and major degradent peaks are passed, it shows that the developed method was stability indicating.

**Table 7: Forced degradation study details**

| Type | Condition &Duration | % Degradation | %Assay | Peak purity |
|---|---|---|---|---|
| Acid | 1N Hcl,24Hrs,50°C | 0.08 | 99.2 | Passes |
| Base | 1N NaoH,24Hrs,50°C | 0.10 | 99.9 | Passes |
| Peroxide | 10% $H_2O_2$,24Hrs,50°C | 37.32 | 62.66 | Passes |
| Diluent | Diluent,24Hrs,50°C | 0.08 | 99.1 | Passes |
| Water | Water,24Hrs,50°C | 0.08 | 99.2 | Passes |
| Thermal | 24Hrs,50°C | 0.09 | 99.0 | Passes |
| Photo | 1.2 million Lux hours | 0.09 | 99.1 | Passes |

**Table 8: System suitability equivalence table**

| Parameter | Pharmacopeia method | UPLC Method |
|---|---|---|
| Standard %RSD | Not more than 10.0 | Not more than 5% |
| RRT | 1)RC-01:0.61<br>2)RC-02:2.55<br>3)RC-03:0.50<br>4)RC-04:4.99 | 1)RC-01:0.68<br>2)RC-02:1.87<br>3)RC-03:0.55<br>4)RC-04:2.22 |
| Response factors | 1)RC-01:1.39<br>2)RC-02:0.83<br>3)RC-03:0.91<br>4)RC-04:0.83 | 1)RC-01:1.39<br>2)RC-02:0.83<br>3)RC-03:0.91<br>4)RC-04:0.83 |
| Critical pair resolution (Between RC-03&RC-01) | 4.732 | 4.798 |

**System suitability results equivalence:**

The difference in the results between developed method and pharmacopeia method was much lesser. The critical pair resolution was maintained above 4.0 in the developed method, it shows that the results are comparable to that of pharmacopeia method results. Shown in table 8.

**API Batch analysis results equivalence:**

The results obtained with the developed method was compared with the pharmacopeia method results, the variation in assay and impurities results was below 0.1%, proves that the method was equivalent to the pharmacopeia method.

**Table 9: API Analysis results equivalence table**

| Details | Pharmacopeia method Results | UPLC Method results |
|---|---|---|
| B.No:SC0091011 | | |
| Known impurity | 10.01% | 0.01% |
| Any unknown individual impurity | 0.02% | 0.02% |
| Total impurity | 0.08% | 0.07% |
| Assay | 100.2% | 100.1% |

**Reference product analysis results equivalence:**

The results obtained with the developed method shows the difference less than 0.05%, shows that the developed method can produce equivalent results to that of pharmacopeia method.

**Table 10: Reference product analysis equivalence table**

| Details | Pharmacopeia method Results | UPLC Method results |
|---|---|---|
| B.No:A236413 | | |
| Known impurity | 0.01% | 0.01% |
| Any unknown individual impurity | 0.05% | 0.05% |
| Total impurity | 0.08% | 0.09% |
| Assay | 100.0 | 99.98 |

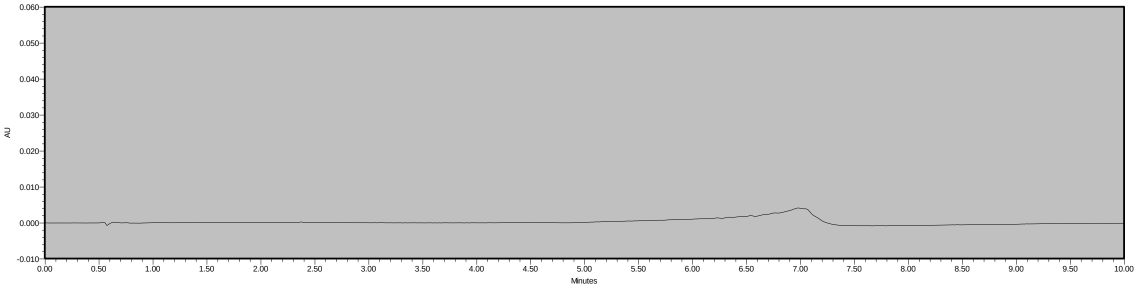

**Figure 3: Blank chromatogram**

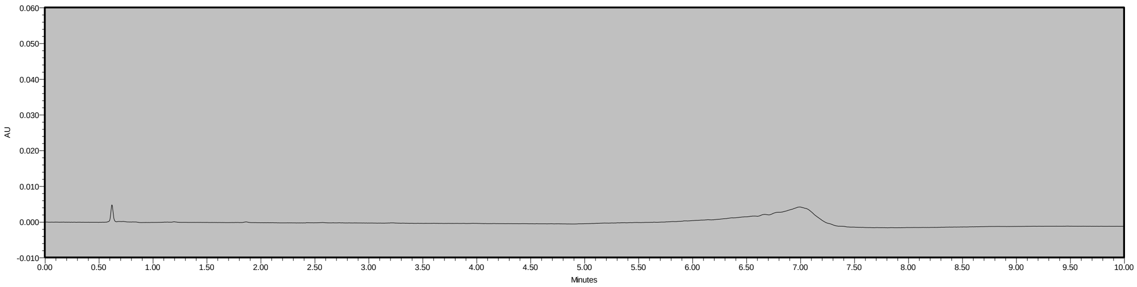

**Figure 4: Placebo chromatogram**

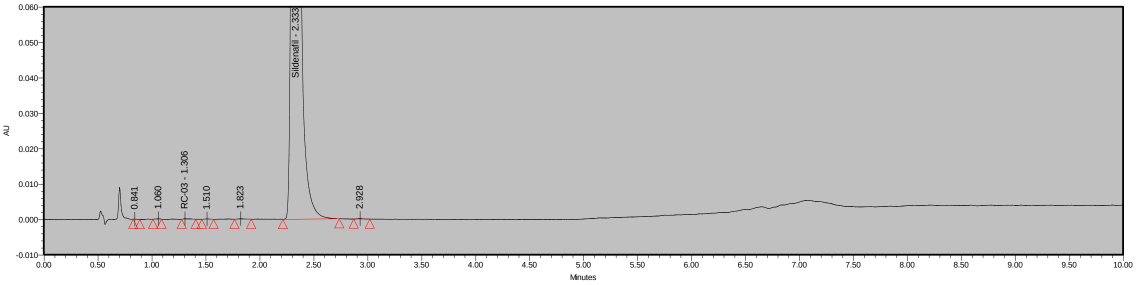

Figure 5: Acid stressed sample chromatogram

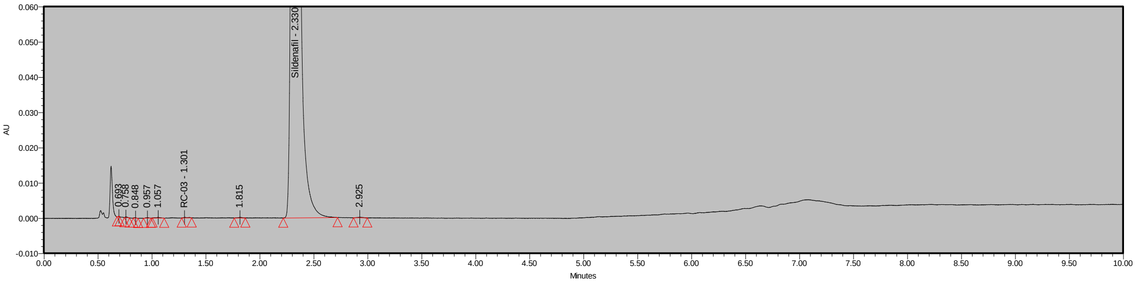

Figure 6: Base stressed sample chromatogram

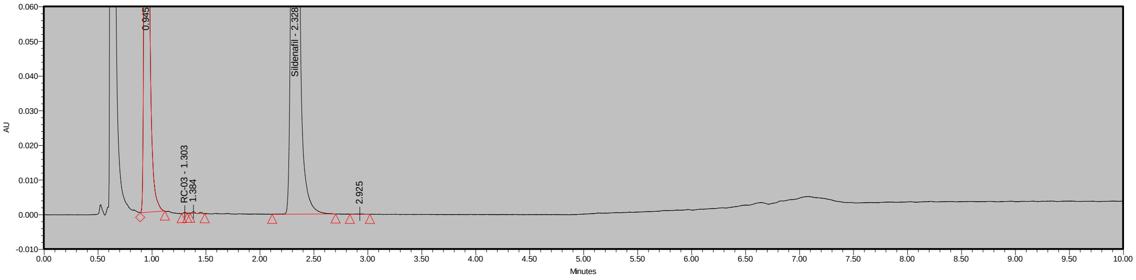

Figure 7: Peroxide stressed sample chromatogram

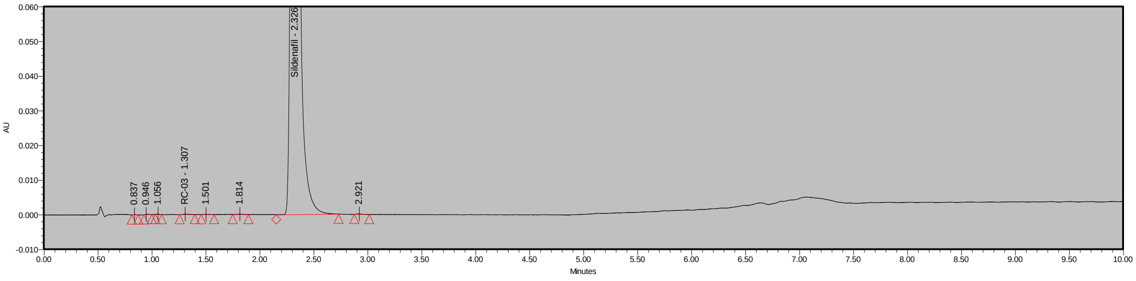

Figure 8: Water stressed sample chromatogram

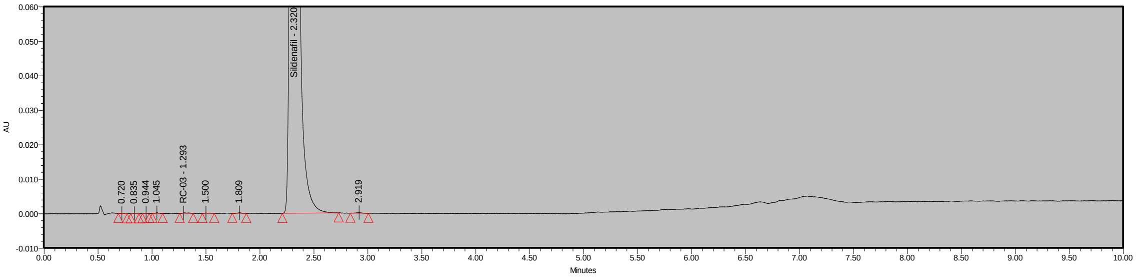

Figure 9: Thermal stressed sample chromatogram

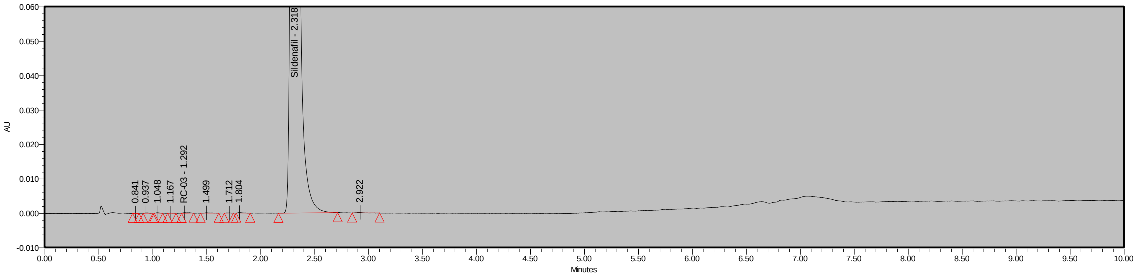

**Figure 10: Light stressed sample chromatogram**

# CONCLUSION:

An UPLC method for related compounds in the commercial drug products and in the tablet formulation was validated in this study. Sildenafil citrate and the other related compounds which may co exist with it as impurities or as degradants gave chromatograms of very well resolved peaks which indicate the specificity of the method and the possibility of using it as an indicator of stability. Slight changes in the experimental conditions did not affect significantiy the resolution of the compounds of interest or their percent recoveries indicating the robustness of the method. All the statistical values (percent recovery, RSD, %, the slope and the intercept, LOD and LOQ) calculated were within the acceptable limits and shown equivalent to the pharmacopeia method. The method can be used for estimation of sildenafil citrate and its related substances in bulk drugs and its tablet dosage forms for quality control purposes.

# ACKNOWLEDGEMENTS

Author thank full to the management of daewoong pharmaceuticals for support of entire work.

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