Method Development and Validation of Quantitative Estimation of Vilazodone Hydrochloride by UV and RP-HPLC

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Abstract : The aim of the study is to develop some new analytical method development and Validation of Quantitative Estimation of Anti-depressant Drug Vilazodone by UV and HPLC was found to be simple, specific, precise, accurate, rapid and economical. The method was developed and validated as per ICH guidelines, concerning accuracy, precision, linearity, ruggedness, limit of detection, limit of quantification and robustness and forced degradation studies. The GRACE ODS phenyl column (4.6 x 150mm,5µm) column was maintained at an ambient temperature and 232 nm λ max conditions. The mixture of di-potassium hydrogen phosphate with buffer (pH 7.4) and methanol in proportion 60:40v/v mobile phase was used in the flow rate of 1 ml/min. All validation methods shows good reproducibility and good recovery. The mean recoveries was found in the range between 99.6-99.9%, with % RSD values were within 2. The limit of detection and limit of quantification were found to be 0.05 µg/ml and 0.01 µg/ml respectively. The method was found to be having suitable application in routine laboratory analysis with high degree of accuracy and precision.

Keywords : VilazodoneHydrochloride, UV, RP-HPLC,Method development and validation.

Introduction:

VilazodoneHClis chemically as 2-benzofurancarboxamide, 5-[4-(4-(5-cyano-1H-indol-3-yl)butyl)-1-piperazinyl]-, hydrochloride (1:1). Its molecular weight is 477.99. The molecular formula is C25H29ClN5O2. Vilazodone HCl is presented in Figure No: 118.Vilazodone tablets are available in 10-mg, 20-mg, and 40-mg strengths. Vilazodone is a novel compound with combined high affinity and selectivity for the 5-hydroxytryptamine (5-HT) transporter and 5HT(1A) receptors12. It has been shown to be equally efficacious as other antidepressants with similar gastrointestinal side effects and possibly with reduced sexual side effects and weight gain. Vilazodone is an antidepressant agent that can used as an alternative for patients who cannot tolerate therapy with other antidepressant classes such as selective

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serotonin reuptake inhibitors or serotonin norepinephrine reuptake inhibitors. Vilazodone increases serotonin levels in the brain by inhibiting the reuptake of serotonin while acting as a partial agonist on serotonin-1A receptors. It has therefore been coined by scientists as a selective partial agonist and reuptake inhibitor (SPARI). The mechanism of the antidepressant effect of vilazodone is not fully understood, but is thought to be related to its enhancement of serotonergic activity in the CNS through selective inhibition of serotonin reuptake. According to literature review (3-13) there are very few methods reported for the determination of Vilazodone in different Instrumental techniques, out of these methods only 1 method was reported by using both UV and RP-HPLC.

Figure

Figure no. 1 Chemical structure of VilazodoneHCl

Materials and Methods:

Equipment: The chromatographic technique performed on an Waters,Empower-2,2695 separation module with a 2487 PDA detector, reversed phase ODS Symmetry C18 (4.6 x 250mm, 5µm, Make: waters) as a stationary phase, AfcosetER- 200A analytical balance, LABINDIA UV 3000+, Adwa – AD 1020 pH meter was used in this study.

Materials: Vilazodone hydrochloride were obtained from Matrix. HPLC Water, Methanol for HPLC and Water for HPLC were obtained from Lichrosolv Merck Mumbai. All other materials used in the study were of analytical or pharmaceutical grade.

Chromatographic Conditions: The sample separation was achieved on an ODS column, symmetry C18 (4.6 x 250mm, 5µm, Make: waters) column, aided by mobile phase mixture of di-potassium hydrogen phosphate with buffer (pH 7.4) and methanol in proportion 60:40v/v. The flow rate was 1 ml/min with the injection volume as 10 µl at ambient temperature and was detected on a UV detector at a wavelength of 232 nm.

Buffer Preparation: weighed about 17.418 grams of K2HPO4 was taken in a 1000ml volumetric flask, dissolved and diluted to 1000ml with HPLC water and the volume was adjusted to pH with Orthophosphoricacid.

Mobile Phase Preparation: Accurately measured 600 ml (60%) of above buffer and 400 ml (40%) of HPLC methanol were mixed and degassed in an ultrasonic water bath for 10 minutes and then filtered through 0.45 µ filter under vacuum filtration.
Preparation of Standard Solutions:

**HPLC Method** - About 50 mg of Vilazodone hydrochloride is weighed and transferred to a 50 ml volumetric flask, it was dissolved with methanol and the volume was made up to the mark with HPLC water. Further 5 ml of above solution was diluted to 100 ml with water. Further 5 ml is diluted to 25 ml with water to get 10 μg/ml Vilazodone hydrochloride.

**UV Method** - Accurately weighed and transferred 10 mg of Vilazodone hydrochloride into a 100 ml volumetric flask add about 70 mL of diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent (Stock solution). Further pipette 0.8 ml of the Vilazodone hydrochloride stock solution into a 10 ml volumetric flask and dilute up to the mark with diluent.

Preparation of Sample Solution:

**HPLC Method** - 20 (Vilazadone) tablets was prepared by grinding them to a fine, uniform size powder. Weight equivalent to 50 mg of Vilazodone was accurately weighed and quantitatively transferred into a 50 ml volumetric flask, it was dissolved with methanol and the volume was made up to the mark with HPLC water. Further 5 ml of above solution was diluted to 100 ml with water. Further 5 ml is diluted to 25 ml with water to get 10 μg/ml Vilazodone hydrochloride.

**UV Method** - Take 20 tablets and crush to powder. Accurately weigh the sample equivalent to 10 mg and transfer into a 100 mL volumetric flask add about 70 mL of diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent (Stock solution). Further pipette 0.8 ml of the Vilazodone hydrochloride stock solution into a 10 ml volumetric flask and dilute up to the mark with diluent.

Diluent Preparation:

**HPLC Method** : Mobile phase was used as the diluent.

**UV Method** : Water was used as the diluent.

Determination of Wave length selection:

UV spectrum of 10 μg/ml vilazodone hydrochloride in diluents (mobile phase composition) was recorded by scanning in the range of 200 nm to 400 nm. From the UV spectrum wavelength selected as 232 nm (Figure no 2). At this wavelength the drug show maximum absorbance.

![UV spectrum of vilazodonehydrochloride](image)
Method validation:

Method validation is to demonstrate that the method is suitable for its intended purpose as it is stated in ICH guidelines. The method was validated for linearity, Correlation coefficient, Method precision, System precision, Intermediate precision, No. of Theoretical plates, accuracy, LOD, LOQ.

Results for UV method:

Validation for the proposed method of UV Spectroscopy:

The developed method was validated based on ICH guidelines with use of UV-Visible spectrophotometer.

Accuracy: Accuracy studies were carried out at 50%, 100% and 150% levels of standard solutions. % RSD was calculated by analyzing each level in triplicate. The results were tabulated (Table no 1).

| Spiked level | Sample Absorbance | µg/ml added | µg/ml found | % Recovery | % Mean recovery |
|--------------|-------------------|-------------|-------------|------------|----------------|
| 50%          | 0.3230            | 1           | 0.9808      | 98.08      |                |
| 50%          | 0.3254            | 1           | 0.9896      | 98.96      |                |
| 50%          | 0.3249            | 1           | 0.9887      | 98.87      | 98.91          |
| 50%          | 0.3285            | 1           | 0.9920      | 99.20      |                |
| 50%          | 0.3280            | 1           | 0.9910      | 99.10      |                |
| 50%          | 0.3292            | 1           | 0.9926      | 99.26      |                |
| 100%         | 0.6578            | 2           | 1.986       | 99.30      |                |
| 100%         | 0.6585            | 2           | 1.989       | 99.45      | 99.28          |
| 100%         | 0.6561            | 2           | 1.982       | 99.10      |                |
| 150%         | 0.9837            | 3           | 3.02        | 100.60     |                |
| 150%         | 0.9844            | 3           | 3.04        | 101.30     |                |
| 150%         | 0.9824            | 3           | 3.01        | 100.30     | 101.16         |
| 150%         | 0.9868            | 3           | 3.08        | 102.60     |                |
| 150%         | 0.9856            | 3           | 3.05        | 101.60     |                |
| 150%         | 0.9834            | 3           | 3.02        | 100.60     |                |

Precision: Precision is the degree of repeatability of an analytical method under normal operational conditions. The precision and accuracy were determined with standard quality control samples (in addition to calibration standards) prepared in triplicate at different concentration levels covering the entire linearity range. The precision of the assay was determined by repeatability (intraday) and intermediate precision (inter-day) and reported as RSD % for a statistically significant number of replicate measurements. The intermediate precision was studied by comparing the assays on three different days and the results are documented as the standard deviation and % RSD (Method precision (Table no 2), System precision (Table no 3), Intermediate precision (Table no 4).
**Table no 02: Results for method precision**

| Reading | Absorbance | Reading | Absorbance |
|---------|------------|---------|------------|
| Reading-1 | 0.6557 | Reading-1 | 0.6566 |
| Reading-2 | 0.6554 | Reading-2 | 0.6564 |
| Reading-3 | 0.6547 | Reading-3 | 0.6562 |
| Reading-4 | 0.6547 | Reading-4 | 0.6566 |
| Reading-5 | 0.6540 | Reading-5 | 0.6265 |
| Reading-6 | 0.6553 | Reading-6 | 0.6263 |
| Average | 0.65495 | Average | 0.6464 |
| Standard deviation | 0.000826 | Standard deviation | 0.000317 |
| % RSD | 0.126 | % RSD | 0.086 |

**Table no 03: Results for system precision**

| Reading | Absorbance | Reading | Absorbance |
|---------|------------|---------|------------|
| Reading-1 | 0.6557 | Reading-1 | 0.6566 |
| Reading-2 | 0.6554 | Reading-2 | 0.6564 |
| Reading-3 | 0.6547 | Reading-3 | 0.6562 |
| Reading-4 | 0.6547 | Reading-4 | 0.6566 |
| Reading-5 | 0.6540 | Reading-5 | 0.6265 |
| Reading-6 | 0.6553 | Reading-6 | 0.6263 |
| Average | 0.65495 | Average | 0.6464 |
| Standard deviation | 0.000826 | Standard deviation | 0.000317 |
| % RSD | 0.126 | % RSD | 0.086 |

**Table no 04: Results for Intermediate precision**

| Reading | Absorbance |
|---------|------------|
| Reading-1 | 0.6554 |
| Reading-2 | 0.6552 |
| Reading-3 | 0.6557 |
| Reading-4 | 0.6552 |
| Reading-5 | 0.6555 |
| Reading-6 | 0.6556 |
| Average | 0.655433 |
| Standard deviation | 0.000207 |
| % RSD | 0.0315 |

**Linearity:** The method was validated according to ICH Q2B guidelines for validation of analytical procedures in order to determine the linearity, sensitivity, precision and accuracy of the analyte. For Vilazodone, five point calibration curves were generated with the appropriate volumes of the working standard solutions for UV methods. Calibration curve & Linearity table is presented in Figure No: 3 & Table No: 5.
Fig no 03: Calibration curve for Vilazodone hydrochloride (UV)

Table no 05: Table linearity results of vilazodone

| CONC% | Absorbance | µg/ml |
|-------|------------|-------|
| 50    | 0.324      | 1     |
| 75    | 0.4962     | 1.50  |
| 100   | 0.6555     | 2.00  |
| 125   | 0.8241     | 2.50  |
| 150   | 0.9812     | 3     |

Range: The analytical range was found to be between 1 to 3 µg/ml for UV-visible spectrophotometer.

LOD & LOQ Values:

The LOD & LOQ were calculated according to the formulas from the calibration graph & reported.

Table no 06: LOD & LOQ results

| Sample                  | LOD(µg/ml) | LOQ(µg/ml) |
|-------------------------|------------|------------|
| Vilazodone hydrochloride| 0.1601     | 0.1146     |

Results for RP-HPLC Method

The developed method was validated based on ICH guidelines which detect and quantitate drug in bulk form with use of HPLC system equipped with PDA detector.
Figure no 4: Shows Chromatogram for Vilazodone hydrochloride

Table no 07: shows Method Development Parameters:

| Retention Time | Area     | % Area | Height | s/n | USP Tailing | USP Plate Count |
|----------------|----------|--------|--------|-----|-------------|-----------------|
| 3.104          | 2628219  | 100.00 | 571605 | 394 | 1.29        | 10877           |

From the above chromatogram it was observed that the Vilazodone hydrochloride was eluted at retention time 3.104. Tailing factor for the peaks due to Vilazodone hydrochloride is not more than 2.0. Theoretical plates for the Vilazodone hydrochloride peak is not less than 2000. The chromatographic parameters such as retention time, No. of theoretical plates and asymmetrical factors were calculated for all chromatograms and shown in table.

Validation for the proposed method of HPLC:

Accuracy: The mean recoveries were found in the range of 99.6-99.9%. The results are presented in table no 8.

Table no 08: Accuracy results for vilazodone hydrochloride (HPLC)

| Sample name | Area     | Amount added in µg/ml | Amount found in in µg/ml | % recovery | Mean recovery |
|-------------|----------|------------------------|--------------------------|------------|--------------|
| 50%         | 1316417  | 5                      | 4.99                     | 99.8       | 99.8         |
| 50%         | 1312470  | 5                      | 4.98                     | 99.6       |              |
| 50%         | 1314429  | 5                      | 4.99                     | 99.8       | 99.9         |
| 50%         | 1316351  | 5                      | 4.99                     | 99.8       |              |
| 50%         | 1317997  | 5                      | 4.97                     | 99.4       |              |
| 50%         | 1315867  | 5                      | 5.08                     | 101.6      |              |
| 100%        | 2631181  | 10                     | 9.98                     | 99.8       | 99.8         |
| 100%        | 2631961  | 10                     | 9.98                     | 99.8       | 99.8         |
| 100%        | 2635290  | 10                     | 9.99                     | 99.9       |              |
| 150%        | 3950109  | 15                     | 14.99                    | 99.9       |              |
| 150%        | 3952582  | 15                     | 14.98                    | 99.8       |              |
| 150%        | 3958125  | 15                     | 14.85                    | 99          | 99.6         |
| 150%        | 3955227  | 15                     | 14.89                    | 99.2       |              |
| 150%        | 3956218  | 15                     | 14.97                    | 99.8       |              |
| 150%        | 3956234  | 15                     | 15.01                    | 100.06     |              |
Precision:

In the Precision study was assessed by injection repeatability tests. For injection repeatability vilazodone hydrochloride is injected in replicate. In this method precision was confirmed by low % RSD values of peak area for all components and reported in table 9, 10 and 11. The % RSD values were within 2 and the method was found to be precise.

Table no 09: Method Precision Results for Vilazodone hydrochloride

| Injection | RT      | Area    |
|-----------|---------|---------|
| Injection-1 | 3.103   | 2630448 |
| Injection-2 | 3.101   | 2634681 |
| Injection-3 | 3.102   | 2633307 |
| Injection-4 | 3.100   | 2631763 |
| Injection-5 | 3.099   | 2633495 |
| Injection-6 | 3.098   | 2631948 |
| Average    | 3.1005  | 2632607 |
| Standard Deviation | 0.001871 | 1509.167 |
| % RSD      | 0.0603  | 0.0573  |

Table no 10: System Precision Results

| Injection | RT      | Area    |
|-----------|---------|---------|
| Injection-1 | 3.103   | 2631948 |
| Injection-2 | 3.101   | 2634681 |
| Injection-3 | 3.100   | 2634578 |
| Injection-4 | 3.100   | 2631763 |
| Injection-5 | 3.101   | 2635607 |
| Injection-6 | 3.098   | 2632684 |
| Average    | 3.1005  | 2633376 |
| Standard Deviation | 0.001871 | 1398.75 |
| % RSD      | 0.0603  | 0.0531  |

Table no 11: ID-Precision Results

| Injection | RT      | Area    |
|-----------|---------|---------|
| Injection-1 | 3.098   | 2631948 |
| Injection-2 | 3.101   | 2634681 |
| Injection-3 | 3.100   | 2631763 |
| Injection-4 | 3.099   | 2633495 |
| Injection-5 | 3.100   | 2631763 |
| Injection-6 | 3.098   | 2631843 |
| Average    | 3.09933 | 2632582 |
| Standard Deviation | 0.001211 | 1227.108 |
| % RSD      | 0.0390  | 0.0466  |

Linearity & Range:

The calibration curves were linear in the range 15-75 µg/ml for Aliskiren hemifumarate, 16-80 µg/ml for Valsartan. The correlation coefficient (‘r’) value was found to be >0.999 for Vilazodone hydrochloride. The results are presented in figure no 5 and table no 12.
**Figure no 5** Calibration graph of Vilazodone Hydrochloride

**Table no. 12: Linearity results for Vilazodone hydrochloride**

| S.No | Linearity Level | CONC% | Area | µg/ml |
|------|----------------|-------|------|-------|
| 1    | I              | 50    | 1318543 | 5     |
| 2    | II             | 75    | 1978842 | 7.50  |
| 3    | III            | 100   | 2632340 | 10.00 |
| 4    | IV             | 125   | 3290035 | 12.5  |
| 5    | V              | 150   | 3959003 | 15    |

**Limit of detection (LOD):**

Limit of detection (LOD) is estimated from the signal-to-noise ratio. The detection limit was defined as the lowest concentration level resulting in a peak height of three times the baseline noise. LOD values of vilazodone hydrochloride is reported in table no 13. The values were found to be within the range.

**Table no 13: LOD results for vilazodone hydrochloride**

| Drug                | LOD               |
|---------------------|-------------------|
| vilazodone hydrochloride | Concentration (µg/ml) | 0.05   |
|                     | Retention time(t<sub>r</sub>) | 3.071min |
|                     | Area              | 30151  |
|                     | Tailing           | 1.297  |
|                     | Pate count        | 9352   |
|                     | s/n               | 3.307  |
Limit of Quantification:

The quantification limit was defined as the lowest concentration level that provided a peak height with a signal-to-noise ratio higher than 10. LOQ values of vilazodone hydrochloride is reported in Table 14. The values were found to be within the range.

Table no 14: LOQ results for Vilazodone hydrochloride

| Drug                     | LOD               |
|--------------------------|-------------------|
| Vilazodonehydrochloride  | Concentration (µg/ml) 0.01 |
|                          | Retention time(t_R) 3.077min |
|                          | Area 78123         |
|                          | Tailing 1.383      |
|                          | Plate count 12638  |
|                          | s/n 10.22          |

Robustness:

Keeping the ratio of mobile phase constant and the chromatograms of drug solution were recorded with different flow rates such as 0.8 ml / min, 1 ml / min and 1.2 ml / min. At the flow rate of 1 ml / min, the peaks were sharp with good resolution and found to be satisfactory. So 1 ml / min flow rate was kept constant throughout the analysis. The results are presented in Table 15. Keeping the flow rate constant (1ml / min) and the chromatograms of drug solution were recorded by changing temperatures. At temperature 20 ºC, 25 ºC, 30 ºC the peaks obtained are observed. By increasing temperature the retention time decreased. But plate count also decreased. The results are presented in Table 16.

Table no. 15 : Study of Robustness results for Vilazodone hydrochloride (Flow Rate)

| S.No. | Flow Rate (ml/min) | RT | AREA   | USP Plate Count | USP Tailing | S/N   |
|-------|--------------------|----|--------|-----------------|-------------|-------|
| 1     | 0.8                | 3.628 | 3296981 | 12085 | 1.345 | 402.707 |
| 2     | 1.0                | 3.104 | 2628219 | 10877 | 1.29  | 1.394  |
| 3     | 1.2                | 2.563 | 2104137 | 9914  | 1.305 | 1.331939|

Table no. 16: Study of Robustness (Effect of Temperature)

| S.No. | Temperature in ºC | RT | AREA   | USP Plate Count | USP Tailing | S/N   |
|-------|-------------------|----|--------|-----------------|-------------|-------|
| 1     | 20 ºC             | 3.076 | 2560365 | 10887 | 1.315 | 342.249 |
| 2     | 25 ºC             | 3.104 | 2628219 | 10877 | 1.29  | 1.394  |
| 3     | 30 ºC             | 3.064 | 2587241 | 10728 | 1.298 | 1.347857|

Forced degradation studies results:

Table no. 17: Degradation studies of vilazodone hydrochloride

| S.No. | Degradation       | Rt   | Area   |
|-------|-------------------|------|--------|
| 1     | Acid degradation  | 3.078 | 2606188 |
| 2     | Base degradation  | 3.021 | 26012343|
| 3     | Thermal degradation | 3.079  | 2612568 |
| 4     | Photolytic degradation | 3.081  | 2615387 |
| 5     | Oxidative degradation | 3.081  | 2625387 |
Table no. 18: Validation summary for Vilazodone hydrochloride

| S.NO | Parameter                      | Acceptance criteria | HPLC       | UV     |
|------|--------------------------------|---------------------|------------|--------|
| 1    | Linearity range(µg/ml)         | -                   | 40-120(µg/ml) | 1-3(µg/ml) |
| 2    | Correlation coefficient        | NLT 0.999           | 0.999      | 0.999  |
| 3    | No.of Theoretical plates       | NLT 2500            | 10877      | -      |
| 4    | Method precision               | %RSD(NMT 2%)        | 0.0573     | 0.126  |
| 5    | System precision               | %RSD(NMT 2%)        | 0.0532     | 0.086  |
| 6    | Intermediate precision         | %RSD(NMT 2%)        | 0.0466     | 0.0315 |
| 7    | % recovery                     | 98-102%             | 99.6-99.9% | 98.91-100.16% |
| 8    | LOD                            | -                   | 0.01(µg/ml) | 0.1061(µg/ml) |
| 9    | LOQ                            | -                   | 0.05(µg/ml) | 0.1146(µg/ml) |

Conclusion:

The proposed Analytical Method Development and Validation of Quantitative Estimation of Anti-depressant Drug Vilazodone by UV and HPLC was found to be simple, specific, precise, accurate, rapid and economical. High performance liquid chromatography is at present one of the most sophisticated tool of the analysis. The estimation of Vilazodone was done by RP-HPLC.. The solutions were chromatographed at a constant flow rate of 1.0 ml/min. The linearity range of Vilazodone were found to be from 5-15 µg/ml. Linear regression coefficient was not more than 0.999. The values of % RSD are less than 2% indicating accuracy and precision of the method. The percentage recovery varies from 98%-102% of Vilazodone. LOD and LOQ were found to be within limit. The Forced degradation studies were performed for acid, base, peroxide, sunlight and temperature. The degradation products do not show any interferences and the method was found to be specific. Simple and rapid UV spectroscopic methods were conveniently developed and validated for the estimation of Vilazodone. The percentage recoveries found using UV-Visible spectroscopy were % and %. UV-Visible spectroscopic analysis for the same samples confirms the results obtained by the former method. Thus HPLC and UV-Visible spectroscopy can be successfully employed for the estimation of vilazadonedrugs in pharmaceutical dosage form. The method was found to be having suitable application in routine laboratory analysis with high degree of accuracy and precision.

References:

1. Available at: https://www.drugbank.ca/drugs/DB06684.
2. Laughren TP, Gobburu J. Vilazodone: clinical basis for the US Food and Drug Administration’s approval of a new antidepressant, The Journal of Clinical Psychiatry. 2011; 72(9): 1166–73.
3. Leslie J. Lovett Gloria A. Nygarda, Shoukry K. W. Khali, A Simple HPLC Method for the Determination of Trazodone Human Serum, Journal of Liquid Chromatography. 1987; 10(5), 909-919.
4. D SreenivasaRao, S Geetha, M.K Srinivasu, G Om Reddy, India. LC determination and purity evaluation of nefazodoneHCl in bulk drug and pharmaceutical formulations; Journal of Pharmaceutical and Biomedical Analysis., 2001;26 (4): 629–636.
5. NevinErk, Turky. Rapid and simple methods for quantitative analysis of some antidepressant in pharmaceutical formulations by using first derivative spectrophotometry and HPLC; Il Farmaco., 2003; 58(12): 1209–121.
6. Laura Mercolini, Carolina Colliva, Mario Amore, Salvatore Fanali, Maria Augusta Raghi, HPLC analysis of the antidepressant trazodone and its main metabolite m-CPP in human plasma; Journal of Pharmaceutical and Biomedical Analysis.,2008; 47(4):882-887.
7. A.E. El Gindy, M. Farouk, O. Abd El Aziz, E.A. Abdullah, Stability Indicating Assay of Trazodone Hydrochloride Using High Performance Liquid Chromatography, Journal of Applied Sciences Research., 2009; 5(11): 2028-2034.
8. Nandini R. Pai, Deeptaunshu Atul Pusalkar, Development and validation of liquid chromatographic method for Trazodone hydrochloride, Journal of Chemical and Pharmaceutical Research., 2010; 2(2): 478-488.

9. Longstreth, J., H. Alcorn Jr., S.K. Swan, Vilazodone pharmacokinetics in subjects with mild to moderate hepatic impairment, Presented at annual meeting of the American Psychiatric Association, New Orleans, LA, May 24, 2010.

10. Yasser A. Z, Rabie S. Farag, M. Elnawawy, Ahmed M. A, Sayde R. AbdAlsalam, Spectrophotometric method for the determination of trazodonehydrochloride in pharmaceutical formulations, International Journal of Pharmaceutical Research., 2011; 2(11):2798-2800.

11. Manavsharma; parikshit R. Jawa; ravinderS. Gill; gulshanbansal. Citalopram hydrobromide: degradation product characterization and a validated stability- indicating LC-UV method, Journal of the Brazilian Chemical Society., 2011; 22(5): 564-571.

12. B. Parameswara Reddy, N. Pramod, P. Venkateswararao, A.M.S. Sudhakarbabu ; Method development and validation for the assay of Vilazodone in bulk and formulation by using RP-HPLC technique, International Journal of Biological & Pharmaceutical Research., 2012; 3(6): 789-795.

13. Ravi Prakash PVDLS, Sumadhuri B, Srikanth M, Validated HPLC-MS/MS Method for Determination of Trazodone in Human Plasma, Open Access Scientific Reports., 2013; 2(2): 1-5.

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