A photo stability indicating HPLC technique for validation of Netupitant and Palonosetron in bulk and formulations

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ABSTRACT

Analytical chemistry is the science that seeks ever improved means of measuring the chemical composition of natural and artificial materials. Netupitant Delayed emesis has been largely associated with the activation of tachykinin family neurokinin 1 receptors. Palonosetron is a selective serotonin 5-HT3 receptor antagonist. The antiemetic activity of the drug is brought about through the inhibition of 5-HT3 receptors present both centrally and peripherally in turn inhibits the visceral afferent stimulation of the vomiting center. The mobile phase used was orthophosphoric and acetate 70% buffer pH 3 and 30% methanol. The assay of Netupitant and Palonosetron was performed with tablets and the % assay was found to be 100.08 and 100.04, The linearity was found to be linear with a correlation coefficient of 0.999, the precision 0.8 and 0.3 for Netupitant and Palonosetron which shows that the method is precise. The validation of developed method shows that the accuracy is well within the limit, which shows that the method is capable of showing good accuracy and reproducibility. The LOD and LOQ for Netupitant were found to be 3.02 and 9.98 and LOD and LOQ for Palonosetron was found to be 3.00 and 10.00. Thus, it shows that the method is stability indicating, sensitive, accurate, robust and precise. Hence, the developed HPLC method can be successfully applied to the pharmaceutical dosage form and can be used for routine analysis.

Keywords: Netupitant, Tachykinin, Palonosetron, Neurokinin, 5-HT3 receptor, HPLC.

INTRODUCTION

Analytical chemistry is the science that seeks ever improved means of measuring the chemical composition of natural and artificial materials [1]. The principle advantage of HPLC compared to classical column chromatography is improved resolution of the separated substance, faster separation times and the increased accuracy, precision and sensitivity [2]. Netupitant is a neurokinin 1 receptor antagonist. Delayed emesis (vomiting) has been largely associated with the activation of tachykinin family neurokinin 1 (NK1) receptors (broadly distributed in the central and peripheral nervous systems) by substance. As shown in vitro and in vivo studies, netupitant inhibits substance P mediated responses [3]. Palonosetron acts as Antiemetic and anti nauseants. Palonosetron is a selective serotonin 5-HT3 receptor antagonist. The antiemetic activity of the drug is brought about through the inhibition of 5-HT3 receptors present both centrally and peripherally (GI tract).

Figure 1: Structure of Netupitant

Figure 2: Structure of Glecaprevir
It has been hypothesized that palonosetron’s potency and long plasma half-life may contribute to its observed efficacy in preventing delayed nausea and vomiting caused by moderately emetogenic cancer chemotherapy [4]. Literature reveals different methods for their analysis in the following formulations [5-8]. But our present plan is to develop a new, simple, precise and accurate method for its analysis in formulation after a detailed study a new RP-HPLC method was decided to be developed and validate it according to ICH guidelines [9] and to study photo stability studies according to ICH Q1B guidelines [10].

MATERIALS AND METHODS

The materials used were HPLC, UV/VIS Spectrophotometer, pH meter, weighing machine, Pipettes, Burettes and Beakers. Nupatrant, Palonosetron KH₂PO₄ Water and Methanol for HPLC. Acetonitrile for HPLC Ortho phosphoric Acid. The Instruments and chemicals used were of HPLC grade.

Wave length selection

UV spectrum of 10µg/ml Netupitant and Palonosetron in diluents (mobile phase composition) was recorded by scanning in the range of 200nm to 400nm. From the UV spectrum wavelength selected as 210nm. At this wavelength both the drugs show good absorbance.

Preparation of mobile phase

Accurately measured 700 ml (70%) of above buffer and 300 ml of Methanol HPLC (30%) were mixed and degassed in an ultrasonic water bath for 10 minutes and then filtered through 0.45µ filter under vacuum filtration.

Preparation of Standard Solution

Accurately weigh and transfer 300 mg of Netupitant and 0.5 mg of Palonosetron working standard into a 10 ml clean dry volumetric flask add about 7 ml of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent.

Further pipette 0.3 ml of the above stock solutions into a 10ml volumetric flask and dilute up to the mark with diluent.

Preparation of Sample Solution

Accurately weigh and transfer 300 mg of Netupitant and 0.5 mg of Palonosetron working standard into a 10 ml clean dry volumetric flask add about 7 ml of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. Further pipette 0.3 ml of the above stock solutions into a 10ml volumetric flask and dilute up to the mark with diluent.

Optimized Method

The column used was YMC 4.6×150mm5µ, Mobile phase consists of 70% buffer pH 3 and 30% methanol, Flow rate was 1.0 ml per min, Wavelength detected at 210 nm, Injected volume was 20µl and Runtime noted as 8min.

Validation of Developed Method

Assay: Inject 20 µl of the standard, sample into the chromatographic system and measure the areas for Netupitant and Palonosetron peaks and calculate the % Assay by using the formulae. 

\[
\text{Assay} = \frac{AT}{AS \times DS \times DT} \times \frac{P}{100} \times \frac{AW}{LC} \times 100
\]

| Drug name     | Label Claim(mg) | % Assay |
|---------------|-----------------|---------|
| Netupitant    | 300             | 100.08  |
| Palonosetron  | 0.5             | 100.04  |

Linearity

Inject each level into the chromatographic system and measure the peak area. Plot a graph of peak area versus concentration (on X-axis concentration and on Y-axis Peak area) and calculate the correlation coefficient. Calibration graphs were shown in (Figure 5, Figure 6) and results are shown in (Table 2).

Precision

The standard solution was injected for six times and measured the area for all six Injections in HPLC. The %RSD for the area of six replicate injections was found to be within the specified limits. The precision results are represented in (Table 3).
Intermediate Precision (Ruggedness)

There was no significant change in assay content and system suitability parameters at different conditions of ruggedness like day to day and system to system variation. The results are represented in (Table 5)

Accuracy

For accuracy determination, three different concentrations were prepared separately i.e. 50%, 100% and 150% for the analyte and chromatograms are recorded for the same.

Calculate the Amount found and Amount added for Netupitant & Palonosetron and calculate the individual recovery and mean recovery values. % recovery was calculated and represented in (Table 6, Table 7).

Limit of Detection for Netupitant and Palonosetron

The LOD and LOQ solutions was prepared injected, for three times and measured the area for all three injections in HPLC. The %RSD for the area of six replicate injections was found to be within the specified limits.

Chromatograms were shown in the (Figure 6) and Results are shown in the (Table 8). The lowest concentration of the sample was prepared with respect to the base line noise and measured the signal to noise ratio.

LOD: It is calculated by using following formula $\frac{3.3}{SD} \times 100$.

Limit of Quantification for Netupitant and Palonosetron

The lowest concentration of the sample was prepared with respect to the base line noise and measured the signal to noise ratio. The chromatograms were recorded as show in (Figure 7) and results are shown in (Table 9).

LOQ: It is calculated by using following formula $\frac{10}{SD} \times 100$

Robustness

The deliberate changes were done in the Flow rate, Mobile Phase composition, Temperature Variation was made to evaluate the impact on the method. The flow rate was varied at 1.1 ml/min to 0.9ml/min. The Organic composition in the Mobile phase was varied from ±10%. The results are shown in (Table 10, Table 11, Table 12, Table 13).

The standard and samples of Netupitant and Palonosetron were injected by changing the conditions of chromatography. There was no significant change in the parameters like resolution, tailing factor, asymmetric factor and plate count.

Results for actual Mobile phase composition have been considered from Accuracy standard. The Retention time, USP plate count, USP tailing factor obtained for change of flow rate, variation in mobile phase was found to be within the acceptance criteria. Hence the method is robust.

Degradation Studies

The International Conference on Harmonization ICH Q1B guideline entitled stability testing of new drug substances and products requires that stress testing be carried out to elucidate the inherent stability characteristics of the active substance. The result of degradation studies is shown in (Table 14).

Preparation of stock

Accurately weigh and transfer 300 mg of Netupitant and 0.5 mg of Palonosetron working standard into a 10 ml clean dry volumetric flask add about 7 mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

Hydrolytic degradation under acidic condition

Pipette 0.3 ml of above solution into a 10ml volumetric flask and 3 ml of 0.1N HCl was added. Then, the volumetric flask was kept at 60ºC for 6 hours and then neutralized with 0.1N NaOH and make up to 10ml with diluent. Filter the solution with 0.22 microns syringe filters and place in vials.

Hydrolytic degradation under alkaline condition

Pipette 0.3ml of above solution into a 10ml volumetric and add 3ml of 0.1N NaOH was added in 10ml of volumetric flask. Then, the volumetric flask was kept at 60ºC for 6 hours and then neutralized with 0.1N HCl and make up to 10ml with diluent. Filter the solution with 0.22 microns syringe filters and place in vials.

Thermal induced degradation

Netupitant and Palonosetron sample was taken in petridish and kept in Hot air oven at 1100 C for 24 hours. Then the sample was taken and diluted with diluents and injected into HPLC and analyzed.

Oxidative degradation

Pipette 0.3ml above stock solution into a 10ml volumetric flask and 1ml of 3% w/v of hydrogen peroxide added in 10 ml of volumetric flask and the volume was made up to the mark with diluent. The volumetric flask was then kept at room temperature for 15 min. Filter the solution with 0.45 microns syringe filters and place in vials.

Photo degradation

Pipette 0.3 ml above stock solution into a 10ml volumetric flask and expose to sunlight for 24hrs and the volume was made up to the mark with diluent. Filter the solution with 0.45 microns syringe filters and place in vials.

RESULTS AND DISCUSSIONS
The estimation of Netupitant and Palonosetron was done by RP-HPLC. The assay of Netupitant and Palonosetron was performed with tablets and the % assay was found to be 100.08 and 100.04. The linearity of Netupitant and Palonosetron was found to be linear with a correlation coefficient of 0.999, the method show precision 0.8 and 0.3 for Netupitant and Palonosetron which shows that the method is precise.

Table 2: Linearity for Netupitant and Palonosetron

| S. No | Linearity Level | Concentration | Area | Concentration | Area |
|-------|----------------|---------------|------|---------------|------|
| 1     | I              | 300           | 30018| 0.5           | 2613 |
| 2     | II             | 600           | 58216| 1             | 4969 |
| 3     | III            | 900           | 86174| 1.5           | 7547 |
| 4     | IV             | 1200          | 117088| 2             | 9909 |
| 5     | V              | 1500          | 147293| 2.5           | 12640|

Correlation Coefficient: 0.999

Table 4: ID Precision results for Netupitant and Palonosetron

| Injection | Area for Netupitant | Area for Palonosetron |
|-----------|---------------------|-----------------------|
| Injection-1 | 86017              | 7508                  |
| Injection-2 | 86172              | 7587                  |
| Injection-3 | 86652              | 7576                  |
| Injection-4 | 86680              | 7534                  |
| Injection-5 | 86818              | 7558                  |
| Injection-6 | 86585              | 7517                  |
| Average    | 86933.8            | 7546.7                |

Standard Deviation: 723.5, 32.1

%RSD: 0.8, 0.4

The intermediate precision is 0.8 and 0.4 for Netupitant and Palonosetron which shows that the method is repeatable when performed in different days also. The total recovery was found to be 100.43% and 100.50% for Netupitant and Palonosetron. The validation of developed method shows that the accuracy is well within the limit, which shows that the method is capable of showing good accuracy and reproducibility.

Table 5: Accuracy (recovery) data for Netupitant and Palonosetron

| % Conc  | Area     | Amount Added (mg) | Amount Found (mg) | % Recovery |
|---------|----------|-------------------|-------------------|------------|
| 50%     | 43148.6  | 10                | 10.01             | 100.43     |
| 100%    | 86625.0  | 20                | 20.09             | 100.43     |
| 150%    | 130313.3 | 30                | 30.23             | 100.43     |
| 50%     | 3818.7   | 5                 | 5.04              | 100.43     |
| 100%    | 7587     | 10                | 10.01             | 100.50     |
| 150%    | 11447    | 15                | 15.10             | 100.50     |

The LOD and LOQ for Netupitant were found to be 3.02 and 9.98 and LOD and LOQ for Palonosetron was found to be 3.00 and 10.00.
Table 6: Flow variations for Netupitant and Palonosetron

| S. No | Flow Rate (ml/min) | Netupitant | Palonosetron |
|-------|--------------------|------------|--------------|
|       |                    | USP Plate Count | USP Tailing | USP Plate Count | USP Tailing |
| 1     | 0.9                | 3962        | 1.17         | 3110           | 1.13        |
| 2     | 1                  | 3914.29     | 1.17         | 3017.92        | 1.13        |
| 3     | 1.1                | 3199.71     | 1.14         | 2675.77        | 1.12        |

Table 7: Organic phase variation for Netupitant and Palonosetron

| S. No | Change in Organic Composition in the Mobile Phase | Netupitant | Palonosetron |
|-------|-------------------------------------------------|------------|--------------|
|       |                                                 | USP Plate Count | USP Tailing | USP Plate Count | USP Tailing |
| 1     | 10% less                                        | 3591       | 1.42         | 2410           | 1.34        |
| 2     | *Actual                                         | 3914.29    | 1.17         | 3017.92        | 1.13        |
| 3     | 10% more                                        | 3340.78    | 1.17         | 3341.82        | 1.18        |

Table 8: Results of LOD and LOQ

| Drug name | Baseline noise(µV) | Signal obtained (µV) | S/N ratio |
|-----------|--------------------|----------------------|-----------|
| Netupitant| 58                 | 175                  | 3.02      |
| Palonosetron | 58                | 174                  | 3.00      |

| Drug name | Baseline noise(µV) | Signal obtained (µV) | S/N ratio |
|-----------|--------------------|----------------------|-----------|
| Netupitant| 58                 | 579                  | 9.98      |
| Palonosetron | 58       | 580                  | 10.00     |

Table 9: Degradation results for Netupitant and Palonosetron

| Sample Name | Netupitant | Palonosetron |
|-------------|------------|--------------|
|             | Area % Degraded | Area % Degraded |
| Standard    | 86056.0     | 7565.7       |
| Acid        | 81872       | 4.86         | 7239        | 4.32         |
| Base        | 81285       | 5.54         | 7298        | 3.54         |
| Peroxide    | 82049       | 4.66         | 7267        | 3.95         |
| Thermal     | 82411       | 4.24         | 7245        | 4.24         |
| Photo       | 82185       | 4.50         | 7264        | 3.99         |

Table 10: Flow variations for Netupitant

| S. No | Flow Rate (ml/min) | System Suitability Results |
|-------|--------------------|----------------------------|
|       |                    | USP Plate Count | USP Tailing |
| 1     | 0.9                | 3962           | 1.17         |
| 2     | 1                  | 3914.29        | 1.17         |
| 3     | 1.1                | 3199.71        | 1.14         |

Table 11: Flow variations for Palonosetron

| S. No | Flow Rate (ml/min) | System Suitability Results |
|-------|--------------------|----------------------------|
|       |                    | USP Plate Count | USP Tailing |
| 1     | 0.9                | 3110           | 1.13         |
| 2     | 1                  | 3017.92        | 1.13         |
| 3     | 1.1                | 2675.77        | 1.12         |

Table 12: Organic phase variation for Netupitant

| S. No | Flow Rate (ml/min) | System Suitability Results |
|-------|--------------------|----------------------------|
|       |                    | USP Plate Count | USP Tailing |
| 1     | 10% less            | 3591           | 1.42         |
| 2     | *Actual             | 3914.29        | 1.17         |
| 3     | 10% more            | 3340.78        | 1.17         |

Table 13: Organic phase variation for Palonosetron

| S. No | Flow Rate (ml/min) | System Suitability Results |
|-------|--------------------|----------------------------|
|       |                    | USP Plate Count | USP Tailing |
| 1     | 10% less            | 2410           | 1.34         |
| 2     | *Actual             | 3017.92        | 1.13         |
| 3     | 10% more            | 3341.82        | 1.18         |
Figure 8: Chromatogram of Netupitant, Palonosetron showing LOQ

The robustness limit for mobile phase variation and flow rate variation are well within the limit, which shows that the method is having good system suitability and precision under given set of conditions.

Table 14: Degradation results

| Sample Name | Netupitant Area | % Degraded | Palonosetron Area | % Degraded |
|-------------|----------------|------------|-------------------|------------|
| Standard    | 161608.8       | 747503     |                   |            |
| Acid        | 153252         | 5.17       | 719259            | 3.78       |
| Base        | 153532         | 5.00       | 719321            | 3.77       |
| Peroxide    | 152239         | 5.80       | 704978            | 5.69       |
| Thermal     | 157522         | 2.51       | 714851            | 4.37       |
| Photo       | 156452         | 3.19       | 714789            | 4.38       |

CONCLUSION

The developed HPLC method and validation was done according to ICH guidelines and photo stability studies were carried out according to ICH Q1B guidelines. Thus, it shows that the method is stability indicating, sensitive, accurate, robust and precise. Hence, the method can be successfully applied to the pharmaceutical dosage form and can be used for routine analysis.

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