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# Analytical Method Development and Validation for Netupitant and Palonosetron in Combine Dosage form by RP-HPLC

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**A B S T R A C T**

High performance liquid chromatography is at present one of the most sophisticated tool for analysis. 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 which shows that the method is useful for routine analysis. The linearity of Netupitant and Palonosetron was found to be linear with a correlation coefficient of 0.999 and 0.999, which shows that the method is capable of producing good sensitivity. The acceptance criteria of precision RSD should not be more than 2.0% and the method show precision 0.8 and 0.3 for Netupitant and Palonosetron which shows that the method is precise. The acceptance criteria of intermediate precision is RSD should not be more than 2.0% and the method shows precision 0.8 and 0.4 for Netupitant and Palonosetron which shows that the method is repeatable when performed in different days also. The accuracy limit is the percentage recovery should be in the range of 97.0% - 103.0%. 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. The acceptance criteria for LOD and LOQ is 3 and 10. The LOD and LOQ for Netupitant was found to be 3.02 and 9.98 and LOD and LOQ for Palonosetron was found to be 3.00 and 10.00. 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.

**Keywords:** RP-HPLC, YMC column, Chromatography, Netupitant and Palonosetron.

**A R T I C L E I N F O**

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**C O N T E N T S**

## 1. Introduction

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 P. Netupitant inhibits substance P mediated responses. Palonosetron is an antiemetic and antinauseants. Palonosetron is a selective serotonin 5-$HT_3$ receptor antagonist. The antiemetic activity of the drug is brought about through the inhibition of 5-$HT_3$ receptors present both centrally (medullary chemoreceptor zone) and peripherally (GI tract). This inhibition of 5-$HT_3$ receptors in turn inhibits the visceral afferent stimulation of the vomiting centre, likely indirectly at the level of the area postrema, as well as through direct inhibition of serotonin activity within the area postrema and the chemoreceptor trigger zone. Alternative mechanisms appear to be primarily responsible for delayed nausea and vomiting induced by emetogenic chemotherapy, since similar temporal relationships between between serotonin and emesis beyond the first day after a dose have not been established, and 5-$HT_3$ receptor antagonists generally have not appeared to be effective alone in preventing or ameliorating delayed effects. 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.

## 2. Methodology

**Instrumentation:**

The instrument used was WATERS HPLC, software - Empower, 2695 separation module, UV detector, LABINDIA UV $3000^{+}$ - UV/VIS spectrophotometer, Adwa – AD 1020 - pH meter, Afcoset ER-200A -Weighing machine, the mobile phase was optimized to potassium dihydrogen phosphate with buffer (pH 3.0), Methanol in proportion 70:30 v/v respectively.

**Materials and reagents:** Netupitant and Palonosetron was provided by PHARMATRAIN, $KH_2PO_4$ was supplied by FINER chemical LTD, Water and Methanol for HPLC was taken from LICHROSOLV (MERCK), Acetonitrile for HPLC was taken from MOLYCHEM, Ortho phosphoric Acid was taken from MERCK.

**Method development:**

A total of five trails were made by changing the mobile phase ratio and pH, Buffer: Methanol $P^H$ 2.5 (30:70 v/v), Buffer: Methanol $P^H$ 2.5 (20:80 v/v), Buffer: Methanol $P^H$ 2.5 (10:90 v/v), Phosphate buffer: Methanol $P^H$ 3.5 (55:45 v/v), 70% buffer pH 3 and 30% methanol.

**Chromatographic conditions:**

Instrument used: Waters HPLC with auto sampler and UV or detector, Temperature: Ambient, Column: YMC 4.6*150mm 5µ, Buffer: 3.4g of $KH_2PO_4$ is taken in 1000 ml water pH adjusted with NaoH, pH: 3.0, Mobile phase: 70% buffer and 30% methanol, Flow rate: 1.0 ml per min, Wavelength: 210 nm, Injection volume: 20 µl, Run time: 8 min.

## 3. Results and Discussion

**System Suitability:**

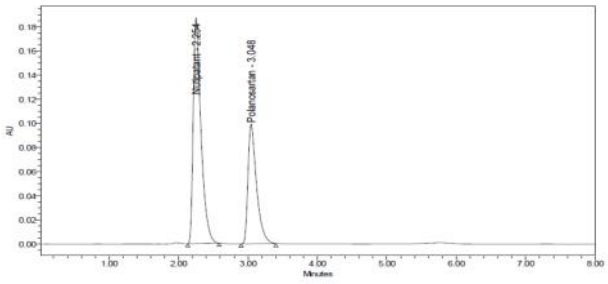


**Figure 1: Chromatogram for system suitability**

**Precision:** Precision of the method was carried out for both sample solutions as described under experimental work. The corresponding chromatogram and results are shown below.

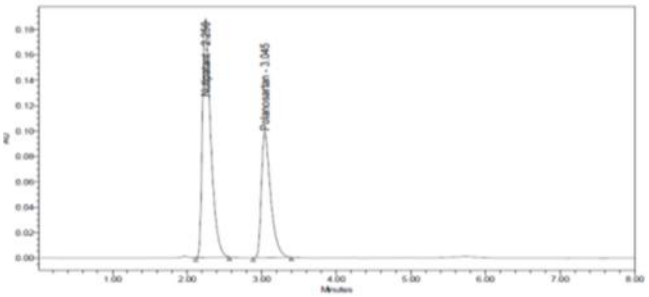


**Figure 2: Chromatogram for Precision**

**Table 1: Results of system suitability parameters**

| S. No | Name | RT (min) | Area (µV sec) | Height (µV) | USP resolution | USP tailing | USP plate count |
|---|---|---|---|---|---|---|---|
| 1 | Netupitant | 2.254 | 86345 | 187425 | | 1.14 | 3930.1 |
| 2 | Palonosetron | 3.059 | 7573 | 103066 | 3.72 | 1.11 | 2910.11 |

**Table 2: Linearity Results: (for Netupitant)**

| S. No | Linearity Level | Concentration | Area |
|---|---|---|---|
| 1 | I | 300 | 30018 |

| | | | |
|---|---|---|---|
| 2 | II | 600 | 58216 |
| 3 | III | 900 | 86174 |
| 4 | IV | 1200 | 117088 |
| 5 | V | 1500 | 147293 |
| Correlation Coefficient | | | 0.999 |

**Table 3: Linearity Results: (for Palonosetron)**

| S. No | Linearity Level | Concentration | Area |
|---|---|---|---|
| 1 | I | 0.5 | 2613 |
| 2 | II | 1 | 4969 |
| 3 | III | 1.5 | 7547 |
| 4 | IV | 2 | 9909 |
| 5 | V | 2.5 | 12640 |
| Correlation Coefficient | | | 0.999 |

**Table 4: Analytical performance parameters of Netupitant and Palonosetron**

| Parameters | Netupitant | Palonosetron |
|---|---|---|
| Slope (m) | 293.42 | 13635 |
| Intercept (c) | 268.8 | 70701 |
| Correlation coefficient ($R^2$) | 0.999 | 0.999 |

**Acceptance criteria:**

- Correlation coefficient ($R^2$) should not be less than 0.999
- The correlation coefficient obtained was 0.999 which is in the acceptance limit.

**Precision:** Precision of the method was carried out for both sample solutions as described under experimental work. The corresponding chromatogram and results are shown below.

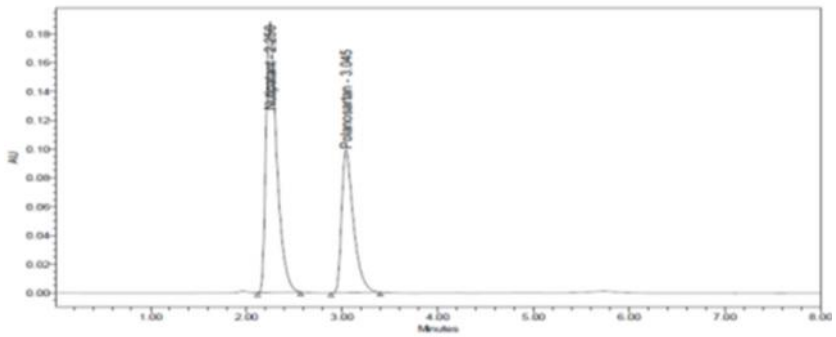


**Figure 4: Calibration graph for Precision**

**Table 5: Precision results for Netupitant and Palonosetron**

| Injection | Area for Netupitant | Area for Palonosetron |
|---|---|---|
| Injection-1 | 87799 | 7524 |
| Injection-2 | 86973 | 7519 |
| Injection-3 | 86232 | 7524 |
| Injection-4 | 87604 | 7581 |
| Injection-5 | 85975 | 7558 |
| Injection-6 | 87018 | 7565 |
| **Average** | 86933.8 | 7545.2 |
| **Standard Deviation** | 723.5 | 26.2 |
| **%RSD** | 0.8 | 0.3 |

- %RSD for sample should be NMT 2
- The %RSD for the standard solution is below 1, which is within the limits hence method is precise.

**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.

**Table 6: 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 |

**Acceptance criteria:**

- %RSD of five different sample solutions should not more than 2
- The %RSD obtained is within the limit, hence the method is rugged.

**Accuracy:** Sample solutions at different concentrations (50%, 100%, and 150%) were prepared and the % recovery was calculated.

**Table 7: Accuracy (recovery) data for Netupitant**

| %Concentration (at specification Level) | Area | Amount Added (mg) | Amount Found (mg) | % Recovery | Mean Recovery |
|---|---|---|---|---|---|
| 50% | 43148.6 | 10 | 10.01 | 100.08 | 100.43 |
| 100% | 86625.0 | 20 | 20.09 | 100.46 | |
| 150% | 130313.3 | 30 | 30.23 | 100.75 | |

**Table 8: Accuracy (recovery) data for Palonosetron**

| %Concentration (at specification Level) | Area | Amount Added (mg) | Amount Found (mg) | % Recovery | Mean Recovery |
|---|---|---|---|---|---|
| 50% | 3818.7 | 5 | 5.04 | 100.75 | 100.50 |
| 100% | 7587 | 10 | 10.01 | 100.08 | |
| 150% | 11447 | 15 | 15.10 | 100.67 | |

**Acceptance Criteria:**

- The percentage recovery was found to be within the limit (97-103%).
- The results obtained for recovery at 50%, 100%, 150% are within the limits. Hence method is accurate.

**Limit of Detection 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.

**Table 9: Results of LOD**

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

- Signal to noise ratio shall be 3 for LOD solution
- The result obtained is within the limit.

**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.

**Table 10: Results of LOQ**

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

- Signal to noise ratio shall be 10 for LOQ solution

**Robustness:** 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.

Table 11: System suitability results 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 12: System suitability results for Palonosetron**

| S. No | Flow Rate (ml/min) | System Suitability Results | | |
|---|---|---|---|---|
| | | USP Plate Count | USP Tailing | USP Resolution |
| 1 | 0.9 | 3110 | 1.13 | 3.60 |
| 2 | 1 | 3017.92 | 1.13 | 3.69 |
| 3 | 1.1 | 2675.77 | 1.12 | 2.66 |

**Table 13: System suitability results for Netupitant**

| S. No | Change in Organic Composition in the Mobile Phase | 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 14: System suitability results for Palonosetron**

| S. No | Change in Organic Composition in the Mobile Phase | System Suitability Results | | |
|---|---|---|---|---|
| | | USP Plate Count | USP Tailing | USP Resolution |
| 1 | 10% less | 2410 | 1.34 | 4.01 |
| 2 | *Actual | 3017.92 | 1.13 | 3.69 |
| 3 | 10% more | 3341.82 | 1.18 | 2.17 |

*****Results for actual Mobile phase composition have been considered from Accuracy standard.

## 4. Conclusion

High performance liquid chromatography is at present one of the most sophisticated tool of the analysis. 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 which shows that the method is useful for routine analysis. The linearity of Netupitant and Palonosetron was found to be linear with a correlation coefficient of 0.999 and 0.999, which shows that the method is capable of producing good sensitivity. The acceptance criteria of precision is RSD should be not more than 2.0% and the method show precision 0.8 and 0.3 for Netupitant and Palonosetron which shows that the method is precise. The acceptance criteria of intermediate precision is RSD should be not more than 2.0% and the method show precision 0.8 and 0.4 for Netupitant and Palonosetron which shows that the method is repeatable when performed in different days also. The accuracy limit is the percentage recovery should be in the range of 97.0% - 103.0%. 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. The acceptance criteria for LOD and LOQ is 3 and 10. The LOD and LOQ for Netupitant was found to be 3.02 and 9.98 and LOD and LOQ for Palonosetron was found to be 3.00 and 10.00. 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.

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