**International Journal of Farmacia**

**Journal Home page: www.ijfjournal.com**

# Method development and validation for simultaneous estimation of Fosnetupitant and Palonosetron by RP-HPLC

**Arshiya*, Ayesha Begum K**

*Department of Pharmaceutical Analysis, Shadan Women's College of Pharmacy, Khairatabad, Hyderabad, India*

**Corresponding Author: Arshiya***

## ABSTRACT

A simple and precise method was developed for estimating fosnetupitant as well as palonosetron. The method was found to be specific and precise. The separation was attained on Xterra RP18 column and linearity was achieved in the concentration rage of 117µg/mL to 470µg/mL of Fosnetupitant, 0.125µg/mL to 0.5µg/mL of Palonosetron with correlation coefficient 0.99. The percent recovery from the assay was found to be 100.19%for fosnetupitant and 100.30% for palonosetron. Limit of detection and quantitation for fosnetupitant and palonosetron were within the acceptable range. From the stability studies, the percentage variation was less than 10.0% which is the desired criteria. Therefore, this method can be adopted to estimate fosnetupitant as well as palonosetron in other pharmaceutical formulations.

**Keywords:** fosnetupitant, palonosetron, HPLC, Method development, Validation.

## INRODUCTION

Fosnetupitant is the pro-drug form of netupitant. Generally, 25% to 30% of patients with a diagnosis of cancer receive chemotherapy as a treatment modality and 70% to 80% of these patients undergoing chemotherapy treatment may experience nausea and vomiting as major side effects. Considered one of the most distressing side effects of chemotherapy, nausea and vomiting has an immense impact on the quality of life of patients receiving certain antineoplastic therapies. Fosnetupitant is taken as an alternative treatment option for patients experiencing chemotherapy-induced nausea and vomiting.[1] Fosnetupitant chloride hydrochloride is white to off-white to yellowish solid or powder. Its solubility is pH dependent: at acidic pH (pH 2), its solubility is 1.4 mg/mL; at basic pH (pH 10), its solubility is 11.5 mg/mL.The fosnetupitant in this drug combination is a selective P/neurokinin-1 (NK-1) receptor antagonist[2-4]. Netupitant, the active moiety of fosnetupitant, is a selective neurokinin 1 (NK1) receptor antagonist with antiemetic activity. Netupitant competitively binds to and blocks the activity of the human substance P/NK1 receptors in the central nervous system (CNS), inhibiting NK1-receptor binding of the endogenous tachykinin neuropeptide substance P (SP), which results in the prevention of chemotherapy-induced nausea and vomiting (CINV). Palonosetron (INN, trade name Aloxi) is an antagonist of 5-HT3 receptors that is indicated for the prevention and treatment of chemotherapy-induced nausea and vomiting (CINV). It is the most effective of the 5-HT3 antagonists in controlling delayed CINV nausea and vomiting that appear more than 24 hours after the first dose of a course of chemotherapy and is the only drug of its class approved for this use by the U.S. Food and Drug Administration. As of 2008, it is the most recent 5-HT3 antagonist to enter clinical use.[5-10]Palonosetron hydrochloride is a white to off-white crystalline powder. It is freely soluble in water, soluble in propylene glycol, and slightly soluble in ethanol and 2-propanol.Palonosetron is a 5-HT3 receptor antagonist with a strong binding affinity for this receptor and little or no affinity for other receptors. Cancer chemotherapy may be associated with a high incidence of nausea and vomiting, particularly when certain agents, such as cisplatin, are used. 5HT3 receptors are located on the nerve terminals of the vagus in the periphery and centrally in the chemoreceptor trigger zone of the area postrema.[11-12] Chemotherapeutic agents produce nausea and vomiting by stimulating the release of serotonin from the enterochromaffin cells of the small intestine. Serotonin then activates 5-HT3 receptors located on vagal afferents to initiate the vomiting reflex. The development of acute emesis is known to depend on serotonin and its 5-HT3 receptors have been demonstrated to selectively stimulate the emetic response.

From the literature survey, it was revealed that few UV spectrophotometric method was developed but were not economical. Moreover, RP-HPLC[13] and LC-MS[14]and derivative methods were also developed which estimates fosnetupitant and palonosetron either individually or in combination. In the present research work, a new method was developed to estimate fosnetupitant and palonosetron simultaneously and validated as per ICH guidelines.[15]

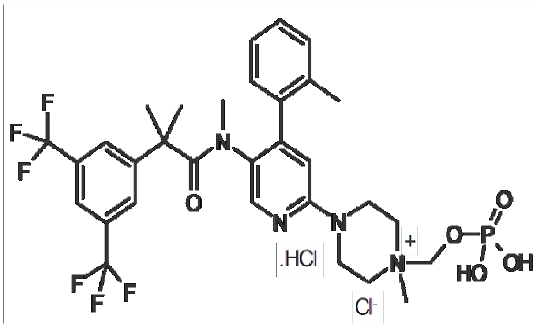


**Figure 1: Structure of fosnetupitant**

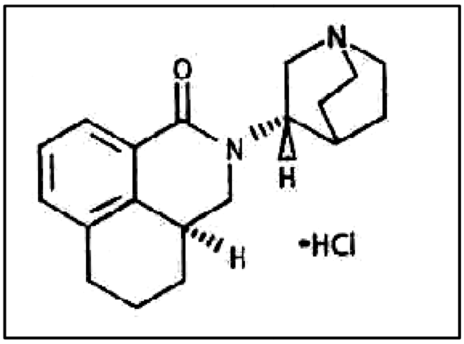


**Figure 2: Structure of palonosetron**

## MATERIALS AND METHODS

**Materials:** Gift samples of fosnetupitant and palonosetron were received from Startech lab, Hyderabad. KH2PO4 was purchased from Final chemicals whereas water, methanol for HPLC and ortho phosphoric acid were purchased from Merck.

**Instrumentation:** Waters HPLC was used for the separation of fosnetupitant and palonosetron. UV/VIS spectrophotometer (LABINDIA UV 12.500$^{+}$) was used for detection. Instruments such as; pH meter used was of Adwa-AD 10100 and weighing machine was of Afcoset ER-1000A.

**Method Development**

**Preparation of Phosphate buffer:** Prepare 800 mL of distilled water in a suitable container. Add 20.214 g of $Na_2HPO_4$-$7H_2O$ to the solution. Add 3.394 g of $NaH_2PO_4H_2O$ to the solution. Adjust solution to final desired pH of 7.4 using NaOH. It was then subjected to filtration through membrane filter followed by sonication for 10mins.

**Mobile phase preparation:** From the above prepared buffer, 700mL is mixed with 300mL of HPLC methanol, mixed, degassed, sonicated for 10min followed by filtration via vacuum filter.

**StockSolution Preparation:** The mixed stock solution was prepared freshly by dissolving 235 mg of fosnetupitant and 0.25 mg of palonosetron in 100 mL of mobile phase.

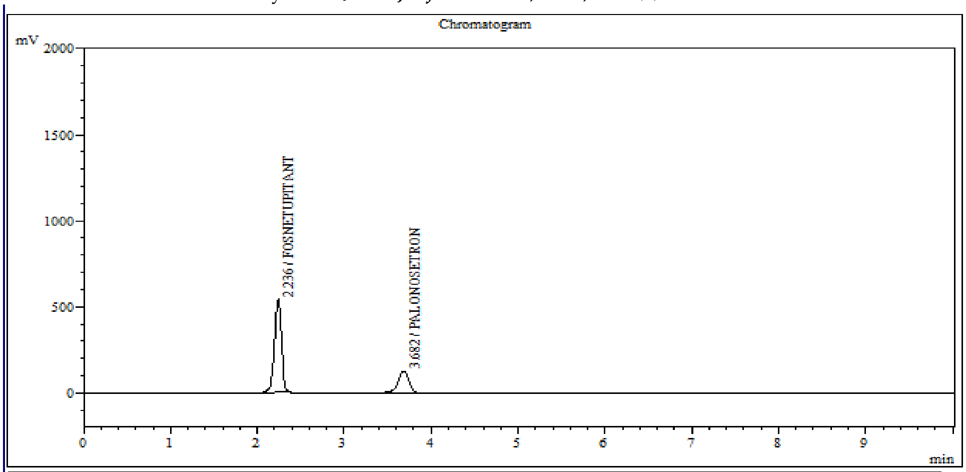
**Standard solution preparation:** 235 mg of fosnetupitant and 0.25 mg of palonosetronworking standard were weighed and added in volumetric flask of 100mL quantity. To it, small quantity of diluent was occasionally added and sonicated and made a final dilution to the mark of the VF using diluent and used as stock solution. From this, 1mL transferred to 10mL VF and diluted using diluents to obtain final volume of 10.0mL.

**Sample solution preparation:** Pipette 1mL of Fosnetupitant and Palonosetron of the above stock solution into a 10mL volumetric flask and dilute up to the mark with Diluents. To it, small quantity of diluent was occasionally added and sonicated and made a final dilution to the mark of the VF using diluent and used as stock solution.

**Procedure:** Mixture of buffer (pH 7.4) and methanol in the ratio 70:30% v/v was used as mobile phase which was injected into the system for 30 minutes prior to injecting the prepared solutions of standard as well as sample. Detection of the drug was achieved at the wavelength of 245nm at 25°C. After several trials, method was optimized followed by validation of the method considering various validation parameters.

## RESULTS AND DISCUSSION

Method development was achieved using Xterra RP18 (4.6 x 150mm, 5.0µm). Mobile phase was mixture of Phosphate buffer and methanol (70:30% v/v). Flow rate (1mL/min) and injection volume (20µl) was set. The peaks obtained had good resolution with the retention time 2.236 and 3.682 for fosnetupitant and palonosetron respectively. Chromatogram of optimized trial is shown in figure 3.




PeakTable

Detector A Ch1 245nm

| Peak# | Name | Ret. Time | Area | Area % | Theoretical Plate# | Resolution | Tailing Factor |
|---|---|---|---|---|---|---|---|
| 1 | FOSNETUPITANT | 2.236 | 2925849 | 73.598 | 3393.025 | 0.000 | 0.986 |
| 2 | PALONOSETRON | 3.682 | 1049610 | 26.402 | 4406.319 | 7.704 | 0.923 |
| Total | | | 3975459 | 100.000 | | | |

**Figure 3: Chromatogram of optimized trial**

**System suitability:** All the parameters were evaluated by performing system suitability studies. The recorded responses for suitability studies are depicted in table 1.

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

| S.No | Name | RT (min) | Area (µV sec) | USP resolution | USP tailing | USP plate count |
|---|---|---|---|---|---|---|
| 1 | Fosnetupitant | 2.236 | 2925849 | | 0.986 | 3393.025 |
| 2 | Palonosetron | 3.682 | 1049610 | 7.704 | 0.923 | 4406.319 |

**Method validation:** Validation of the method was evaluated for various parameters which include linearity, specificity, robustness and stability. The method was also evaluated for specificity of the method and was found to be specific as there were no interactions found. Linearity obtained was shown to have good correlation as shown in table 2.

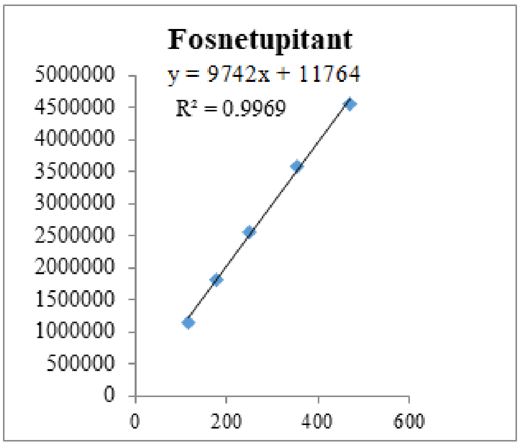
**Table 2: Results of assay**

| | Label Claim (mg) | % Assay |
|---|---|---|
| **Fosnetupitant** | 235 | 100.19 |
| **Palonosetron** | 0.25 | 100.30 |

**Linearity:** The linearity range was observed from 117µg/mL to 470µg/mL of Fosnetupitant, 0.125µg/mL to 0.5µg/mL of Palonosetron. The respective absorbance values are depicted in table 3. The linearity graph plotted is presented in figure 4 and 5 for Fosnetupitantas well as Palonosetron respectively.

**Table 3:Linearity results**

| S. No. | Fosnetupitant | | Palonosetron | |
|---|---|---|---|---|
| | **Concentration (µg/mL)** | **Area** | **Concentration (µg/mL)** | **Area** |
| 1 | 117 | 1144247 | 0.125 | 386439 |
| 2 | 176 | 1812362 | 0.187 | 622922 |
| 3 | 235 | 3062738 | 0.250 | 1090765 |
| 4 | 352 | 4069834 | 0.375 | 1351741 |
| 5 | 470 | 4544205 | 0.500 | 1672640 |
| Slope (m) | | 9742 | 2405 | |
| Intercept (c) | | 11764 | 7894 | |
| Correlation coefficient ($R^2$) | | 0.9969 | 0.9972 | |



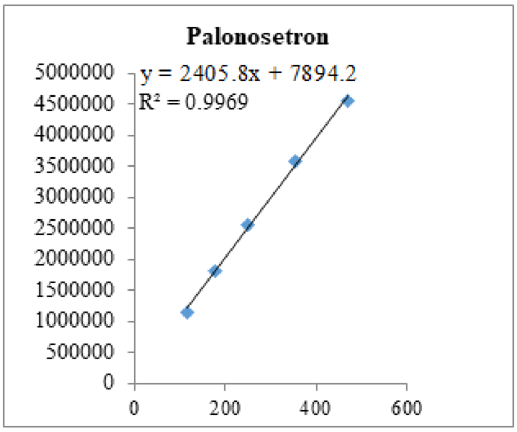




**Figure 4: Linearity graph for FosnetupitantFigure 5: Linearity graph for Palonosetron**

**Accuracy:** Percent recovery of sample solutions at different concentrations (50%, 100%, and 150%) was calculated. The Percent recovery of fosnetupitant and palonosetron are depicted in table 4 and 5 respectively.

**Table 4: Accuracy (recovery) data for Fosnetupitant**

| % Concentration | Area | Added amount (mg) | Amount Found (mg) | PercentRecovery | Mean Recovery |
|---|---|---|---|---|---|
| 50% | 1144247 | 117.0 | 117.22 | 100.18 | 100.17 |
| 100% | 3062738 | 235.0 | 235.46 | 100.19 | |
| 150% | 4544205 | 470 | 470.66 | 100.14 | |

**Table 5: Accuracy (recovery) data for Palonosetron**

| %Concentration | Area | Added amount (mg) | Amount Found (mg) | % Recovery | Mean Recovery |
|---|---|---|---|---|---|
| 50% | 386439 | 0.125 | 0.126 | 100.8 | 100.5 |
| 100% | 1090765 | 0.250 | 0.2512 | 100.3 | |
| 150% | 1672640 | 0.500 | 0.502 | 100.4 | |

**Precision:** Precision of the method was performed for both sample solutions as described under experimental work. The same method was performed on the other day for intermediate precision. The results are depicted in the table 6 and 7.

**Table 6: Results of precision**

| Injection | Area (Fosnetupitant) | Area (Palonosetron) |
|---|---|---|
| Injection-1 | 2855391 | 1024050 |
| Injection-2 | 2853669 | 1023557 |
| Injection-3 | 2930368 | 1049612 |
| Injection-4 | 2916908 | 1041245 |
| Injection-5 | 2925849 | 1049610 |
| **Average** | 2896437 | 1037615 |
| **Standard Deviation** | 20566 | 7723 |
| **%RSD** | 0.71 | 0.74 |

**Table 7: Results of intermediate precision**

| Injection | Area (Fosnetupitant) | Area (Palonosetron) |
|---|---|---|
| Injection-1 | 2853414 | 1023822 |
| Injection-2 | 2951094 | 1059836 |
| Injection-3 | 2925582 | 1056103 |
| Injection-4 | 2916466 | 1041407 |
| Injection-5 | 2967660 | 964840 |

| | | |
|---|---|---|
| Injection-6 | 2973564 | 1035268 |
| **Average** | 2931297 | 1030213 |
| **Standard Deviation** | 14319 | 5679 |
| **%RSD** | 0.48 | 0.55125 |

**Limit of Detection and Quantitation:** Lowest concentrations of the sample were prepared and measured the signal/ noise ratio. Chromatogram for LOD and LOQ is presented in figure 6 and 7 and the results are depicted in table 8.

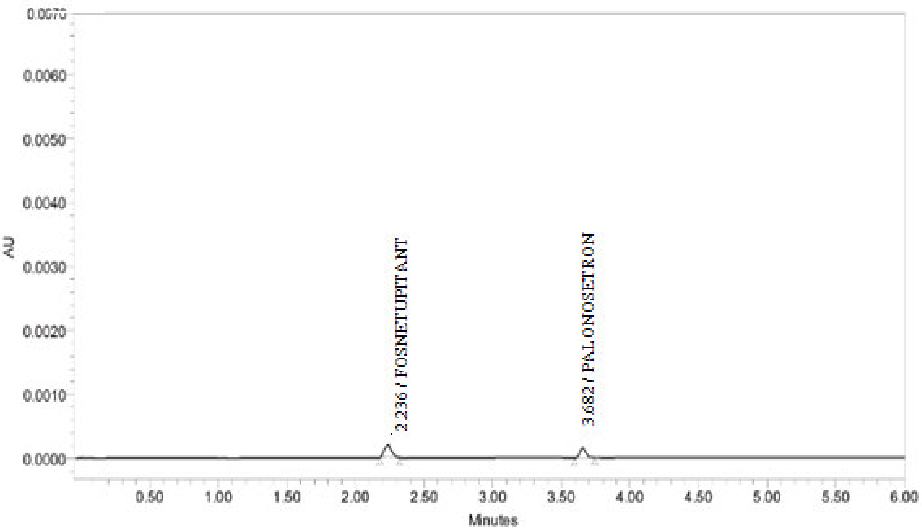



**Figure 6: Chromatogram of Fosnetupitant, Palonosetron showing LOD**

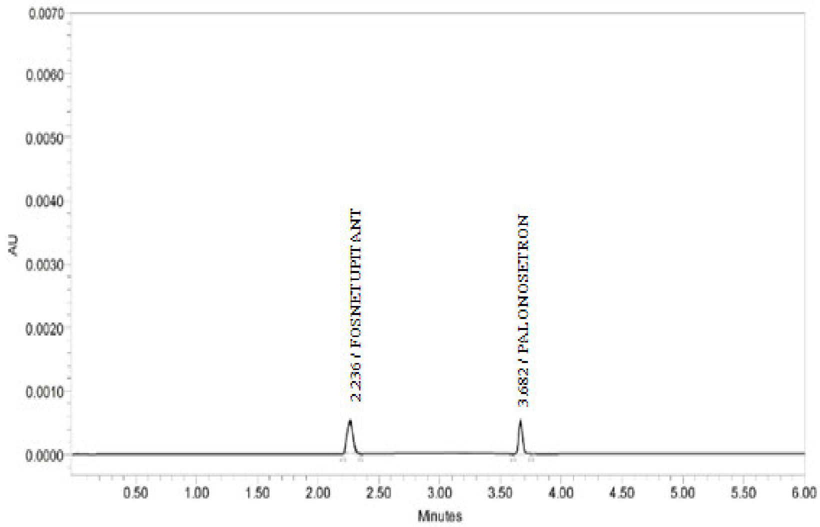


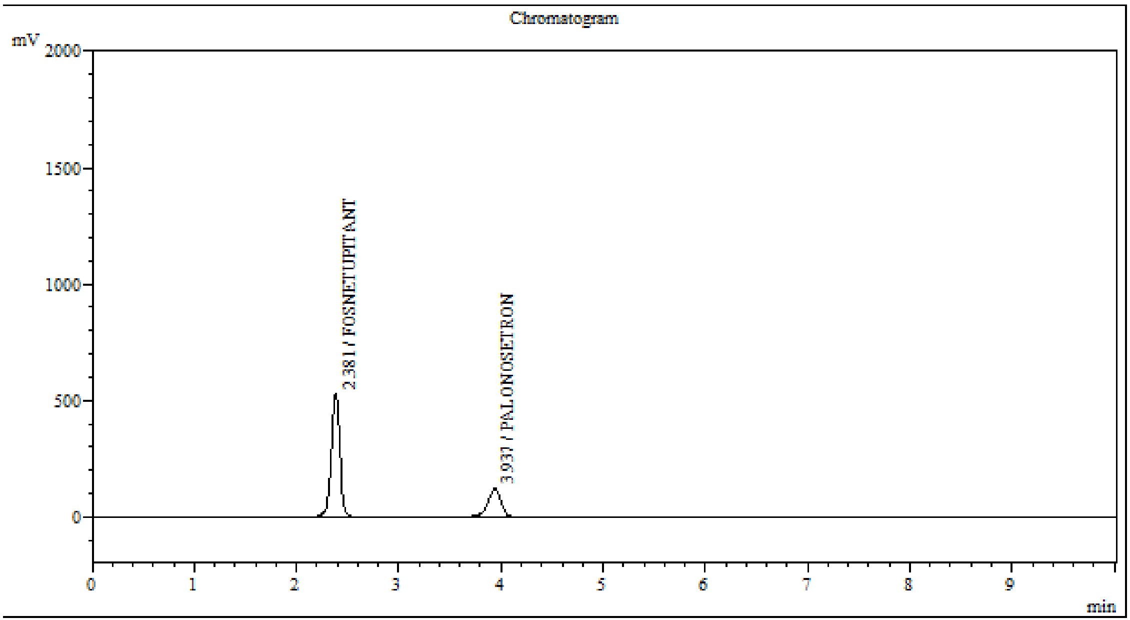

**Figure 7: Chromatogram of Fosnetupitant, Palonosetron showing LOQ**

**Table 8: Results of LOD and LOQ**

| Drug name | Baseline noise (µV) | Signal obtained (µV) | S/N ratio |
|---|---|---|---|
| **Limit of detection** | | | |
| Fosnetupitant | 58 | 172 | 2.97 |
| Palonosetron | 58 | 170 | 2.93 |
| **Limit of quantitation** | | | |
| Fosnetupitant | 58 | 578 | 9.97 |
| Palonosetron | 58 | 577 | 9.95 |

**Robustness:** The standard and samples of both drugs were injected by changing the conditions of chromatography. There was no change observed in the parameters like tailing factor, resolution, plate count and asymmetric factor. Chromatograms for variation in flow rate are presented in figure 8 and 9 whereas chromatograms for variation in composition are presented in figure 10 and 11. Their respective results are depicted in table 9-12

**Variation in flow**

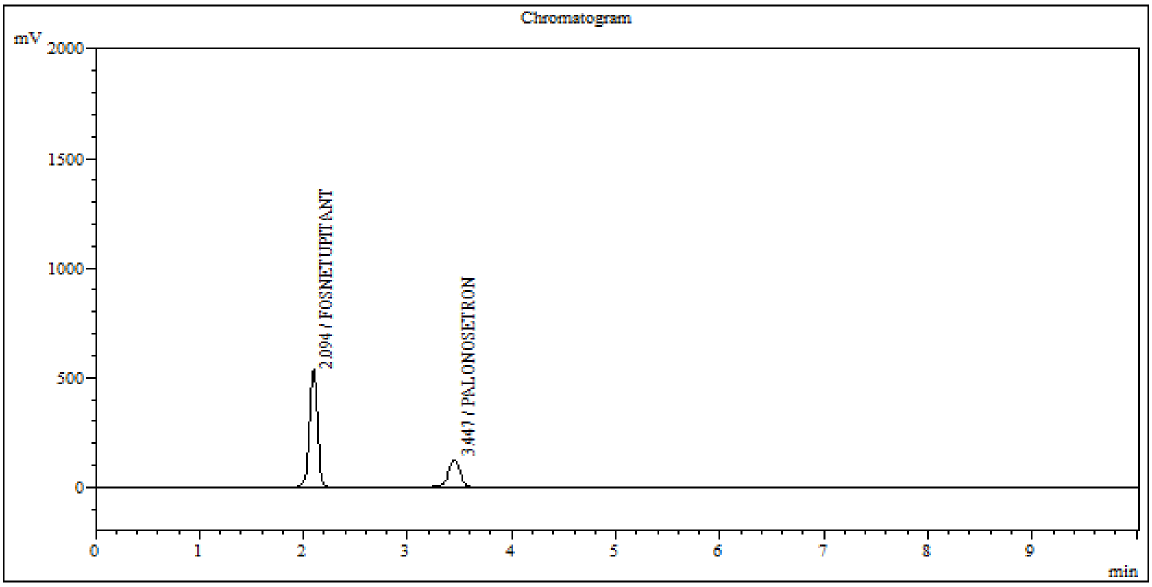



PeakTable

Detector A Ch1 245nm

| Peak# | Name | Ret. Time | Area | Area % |
|---|---|---|---|---|
| 1 | FOSNETUPITANT | 2.381 | 3030699 | 74.127 |
| 2 | PALONOSETRON | 3.937 | 1057808 | 25.873 |
| Total | | | 4088507 | 100.000 |

**Figure 8: Chromatogram showing less flow**



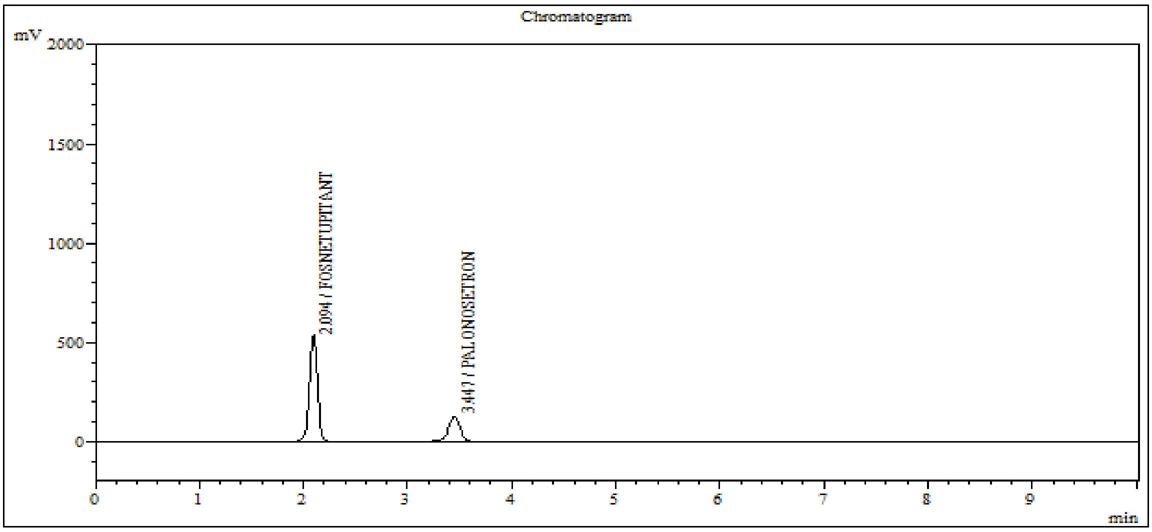

PeakTable

Detector A Ch1 245nm

| Peak# | Name | Ret. Time | Area | Area % |
|---|---|---|---|---|
| 1 | FOSNETUPITANT | 2.094 | 2823210 | 73.934 |
| 2 | PALONOSETRON | 3.447 | 995366 | 26.066 |
| Total | | | 3818576 | 100.000 |

**Figure 9: Chromatogram showing more flow**

**Variation of mobile phase organic composition**

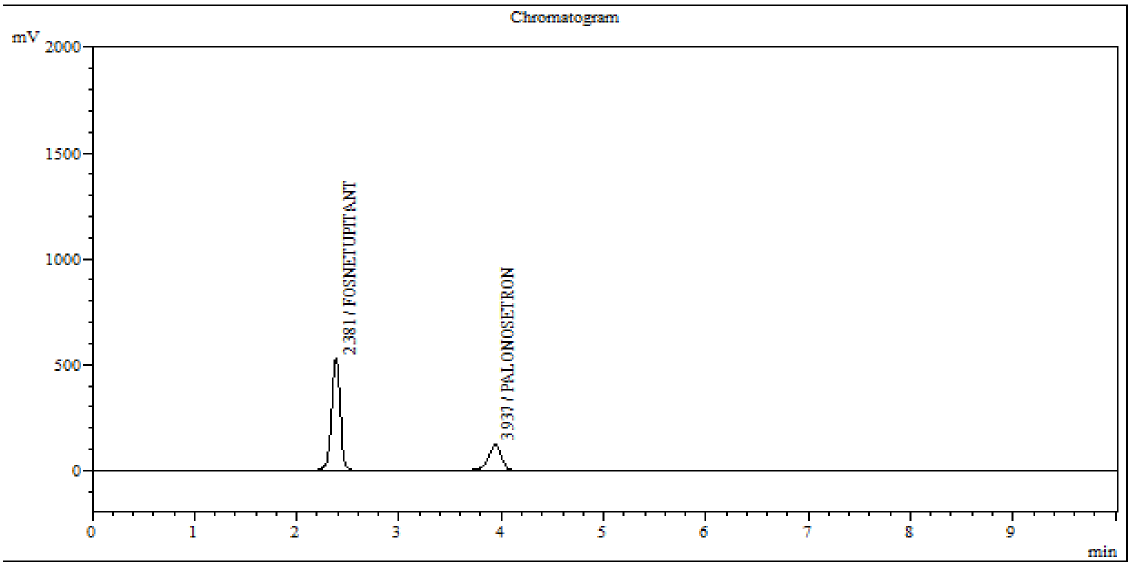



PeakTable

Detector A Ch1 245nm

| Peak# | Name | Ret. Time | Area | Area % |
|---|---|---|---|---|
| 1 | FOSNETUPITANT | 2.094 | 2823210 | 73.934 |
| 2 | PALONOSETRON | 3.447 | 995366 | 26.066 |
| Total | | | 3818576 | 100.000 |

**Figure 10: Chromatogram with less organic composition**




PeakTable

Detector A Ch1 245nm

| Peak# | Name | Ret. Time | Area | Area % |
|---|---|---|---|---|
| 1 | FOSNETUPITANT | 2.381 | 3030699 | 74.127 |
| 2 | PALONOSETRON | 3.937 | 1057808 | 25.873 |
| Total | | | 4088507 | 100.000 |

**Figure 11: Chromatogram with more organic composition**

**Table 9: Results for variation in flow for Fosnetupitant**

| S. No | Flow Rate (mL/min) | System Suitability Results | |
|---|---|---|---|
| | | USP Plate Count | USP Tailing |
| 1 | 0.9 | 2497.32 | 1.39 |
| 2 | 1.0 | 2491.44 | 1.39 |
| 3 | 1.1 | 2501.26 | 1.39 |

**Table 10: Results for variation in flow for Palonosetron**

| S. No | Flow Rate (mL/min) | System Suitability Results | |
|---|---|---|---|
| | | USP Plate Count | USP Tailing |
| 1 | 0.9 | 2768.69 | 1.61 |
| 2 | 1.0 | 2736.33 | 1.61 |
| 3 | 1.1 | 2789.96 | 1.60 |

* Actual flow (1.0mL/min) was considered from Assay standard.

**Table 11: Results for variation in mobile phase composition for Fosnetupitant**

| S. No | Change in Organic Composition in the Mobile Phase | System Suitability Results | |
|---|---|---|---|
| | | USP Plate Count | USP Tailing |
| 1 | 10% less | 2468.33 | 1.40 |
| 2 | *Actual | 2491.44 | 1.39 |
| 3 | 10% more | 2465.45 | 1.41 |

**Table 12: Results for variation in mobile phase composition for Palonosetron**

| S. No | Change in Organic Composition in the Mobile Phase | System Suitability Results | |
|---|---|---|---|
| | | USP Plate Count | USP Tailing |
| 1 | 10% less | 2759.45 | 1.62 |
| 2 | *Actual | 2736.33 | 1.61 |
| 3 | 10% more | 2726.34 | 1.61 |

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

## CONCLUSION

From the above experimental results and parameters it was concluded that, this newly developed method for the simultaneous estimation ofFosnetupitant and Palonosetron was found to be simple, precise, accurate and high resolution and shorter retention time makes this method more acceptable and cost effective and it can be effectively applied for routine analysis in research institutions, quality control department in industries, approved testing laboratories, bio-pharmaceutical and bio-equivalence studies and in clinical pharmacokinetic studies in near future.

## ACKNOWLEDGEMENT

The authors are thankful to Shadan women's college of pharmacy to undergo the research work.

## REFERENCES

1. Akynzeo: Summary of Product Characteristics. European Medicines Agency. Retrieved 12 July 2016.
2. Navari RM: Profile of netupitant/palonosetron (NEPA) fixed dose combination and its potential in the treatment of chemotherapy-induced nausea and vomiting (CINV). Drug Des DevelTher. 2014 Dec 17; 9:155-61.
3. Coyne JW: The First Oral Fixed-Dose Combination of Netupitant and Palonosetron for the Treatment of Chemotherapy-Induced Nausea and Vomiting. J Adv Pract Oncol. 2016 Jan-Feb;7(1):66-70.
4. PierluigiRossetto, Peter MacDonald, Ettore Bigatti, Gaia Banfi, Dario Tentorio, "Processes for preparing palonosetron salts." U.S. Patent US20080200681, issued August 21, 2008.
5. De Leon A: Palonosetron (Aloxi): a second-generation 5-HT (3) receptor antagonist for chemotherapy-induced nausea and vomiting. Proc (Bayl Univ Med Cent). 2006 Oct;19(4):413-6.
6. Stoltz R, Cyong JC, Shah A, Parisi S: Pharmacokinetic and safety evaluation of palonosetron, a 5-hydroxytryptamine-3 receptor antagonist, in U.S. and Japanese healthy subjects. J Clin Pharmacol. 2004 May;44(5):520-31.
7. Rubenstein EB: Palonosetron: a unique 5-HT3 receptor antagonist indicated for the prevention of acute and delayed chemotherapy-induced nausea and vomiting. Clin Adv Hematol Oncol. 2004 May;2(5):284-9.
8. Yang LP, Scott LJ: Palonosetron: in the prevention of nausea and vomiting. Drugs. 2009 Nov 12;69(16):2257-78.
9. Siddiqui MA, Scott LJ: Palonosetron. Drugs. 2004;64(10):1125-32.
10. Eisenberg P, MacKintosh FR, Ritch P, Cornett PA, Macciocchi A: Efficacy, safety and pharmacokinetics of palonosetron in patients receiving highly emetogenic cisplatin-ba.sed chemotherapy: a dose-ranging clinical study. Ann Oncol. 2004 Feb;15(2):330-7.
11. Stoltz R, Parisi S, Shah A, Macciocchi A: Pharmacokinetics, metabolism and excretion of intravenous [l4C]-palonosetron in healthy human volunteers. Biopharm Drug Dispos. 2004 Nov;25(8):329-37.

12. G Karuna, R.V. Valli Kumari, M. Sathish Kumar, S Markatham. Simultaneous method development and validation for estimation of fosnetupitant and palonosetron using HPLC. Int. J. of Pharmacy and Analytical Research Vol-8(4) 2019, 503-517.
13. Arshiya Kousar and M. A. Haneef. RP-HPLC method development and validation for the simultaneous estimation of fosnetupitant and palonosetron in bulk and combined formulation. World Journal of Pharmaceutical Research, 2020, Volume 9, Issue 4, 1129-1148.
14. ICH Q2B guideline (1996), Validation of Analytical Procedures: Methodology, Guidance for Industry, 1-10.