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# Development and Validation of a Stability-Indicating RP-HPLC Method for the Simultaneous Determination of Palonosetron and Fosnetupitant

**G. Sai Charan[1]*, M. Akiful Haque[1]**

[1]School of Pharmacy, Anurag University, Hyderabad, Telangana, India.

*Corresponding Author - saicharanpharmacy@anurag.edu.in

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## ABSTRACT

A simple, accurate, and robust RP-HPLC method was developed and validated for the simultaneous estimation of Palonosetron (PST) and Fosnetupitant (FST) in *Akynzeo I.V.* formulation. Chromatographic separation was achieved using a Waters C18 column (250 × 4.6 mm, 5 µm) with a mobile phase of K2HPO4 buffer: Acetonitrile (70:30 v/v) at a flow rate of 1.0 mL/min, and a detection wavelength of 228 nm. PST and FST exhibited retention times of 1.76 min and 2.31 min, respectively. Linearity was observed within 0.125–0.375 µg/mL for PST and 117.5–352.5 µg/mL for FST. The developed method was validated as per ICH Q2(R1) guidelines and was found precise, accurate, specific, and stability-indicating.

## INTRODUCTION

Palonosetron (PST) and Fosnetupitant (FST) are widely used in combination therapy for the prevention of chemotherapy-induced nausea and vomiting (CINV), a distressing side effect that severely affects patient quality of life during cancer treatment. Palonosetron, a second-generation 5-HT3 receptor antagonist, exhibits high receptor affinity and an extended plasma half-life, providing long-lasting antiemetic effects compared to first-generation agents. Fosnetupitant, a selective neurokinin-1 (NK1) receptor antagonist and prodrug of netupitant, complements the antiemetic action of Palonosetron by blocking the binding of substance P at NK1 receptors. Their combination, marketed as *Akynzeo I.V.*, offers synergistic efficacy by targeting dual emetic pathways—serotonergic and neurokinin-mediated[1-4].

Accurate quantification of these drugs in pharmaceutical dosage forms is essential for ensuring therapeutic effectiveness, dosage consistency, and patient safety. Analytical methods reported in the literature for Palonosetron and related compounds have primarily focused on single-drug analysis using HPLC or UV spectrophotometry. However, simultaneous estimation of Palonosetron and Fosnetupitant poses analytical challenges due to differences in polarity, solubility, and UV absorption characteristics. Limited methods exist for their concurrent determination, especially in parenteral formulations[5-8].

High-Performance Liquid Chromatography (HPLC) is the preferred technique for routine quality control because of its sensitivity, selectivity, and reproducibility. The objective of this study was to develop and validate a simple, precise, and stability-indicating RP-HPLC method for simultaneous estimation of Palonosetron and Fosnetupitant in *Akynzeo I.V.*. The developed method aimed to achieve sharp, well-resolved peaks with minimal run time, while fulfilling the analytical performance parameters specified by the International Council for Harmonisation (ICH) guidelines Q2(R1)[8-12].

## MATERIALS AND METHODS

Chemicals and Reagents

Palonosetron and Fosnetupitant reference standards were procured as pure samples. *Akynzeo I.V.* (containing 0.25 mg PST and 235 mg FST) was used as the commercial formulation. Analytical-grade acetonitrile, phosphoric acid, sodium hydroxide, hydrochloric acid, and hydrogen peroxide were used[13-15].

Instrumentation

A Waters Alliance HPLC system with a photodiode array (PDA) detector was used. Data were processed using Empower software.

**Chromatographic Conditions**

| Parameter | Condition |
|---|---|
| Column | Waters C18 (250 × 4.6 mm, 5 µm) |
| Mobile phase | K2HPO4 buffer: acetonitrile (70:30 v/v) |
| Flow rate | 1.0 mL/min |
| Detection wavelength | 228 nm |
| Injection volume | 10 µL |
| Run time | < 3 min |

**Preparation of Standard Solution[16]**

0.25 mg of PST and 235 mg of FST were dissolved in 10 mL of acetonitrile, sonicated for 10 minutes, and made up to 100 mL with water. 1 mL of this stock was diluted to 10 mL with the diluent.

**Preparation of Sample Solution**

Tablet powder equivalent to 0.25 mg PST and 235 mg FST was transferred to a 100 mL volumetric flask, dissolved in 10 mL acetonitrile, sonicated for 20 minutes, and made up with water.

**Validation Parameters**

The method was validated for linearity, accuracy, precision, robustness, LOD, LOQ, selectivity, and stability according to ICH guidelines[16-19].

Forced Degradation Studies

| Condition | Stress Type | Exposure Time | | Observation |
|---|---|---|---|---|
| 0.1 N HCl | Acidic | 60°C, min | 30 | Clear, stable |
| 0.1 N NaOH | Alkaline | 60°C, min | 30 | Clear, stable |
| 30% H2O2 | Oxidative | 60°C, min | 30 | No major degradation |
| Sunlight | Photolytic | 6 hrs | | Clear, stable |
| 105°C | Thermal | 30 min | | Minor degradation observed |

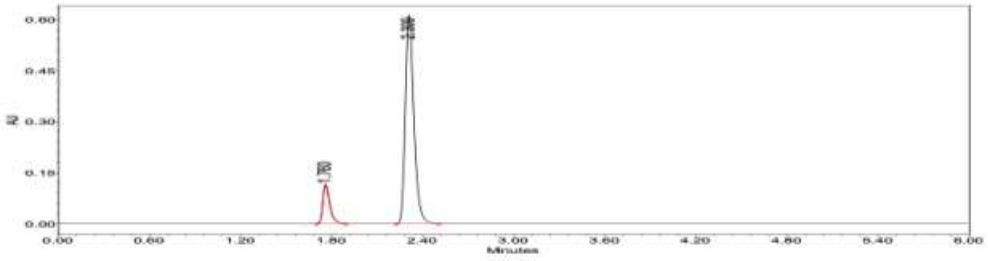


**Figure 1: Chromatogram of PST and FST under optimized conditions.**

**System Suitability**

System suitability parameters were determined to verify performance. Theoretical plates, tailing factor, and resolution were within acceptable limits, ensuring column efficiency and reproducibility. The theoretical plate count exceeded 4000 for both drugs, and tailing factors were less than 1.5, indicating excellent peak symmetry. The resolution between PST and FST was greater than 2.0, demonstrating sufficient separation for simultaneous quantification.

**RESULTS AND DISCUSSION**

Method Development

Several chromatographic conditions were evaluated to achieve optimal separation, resolution, and peak shape. Different mobile phase compositions, flow rates, and buffer pH levels were tested to improve sensitivity and selectivity for both drugs. The optimized method using K2HPO4 buffer: Acetonitrile (70:30 v/v) produced sharp, symmetric peaks with minimal tailing and good baseline resolution. Retention times of 1.76 min for Palonosetron (PST) and 2.309 min for Fosnetupitant (FST) confirmed adequate separation within a short run time (<3 minutes), enhancing throughput and efficiency[20-23].

**Linearity and Calibration**

Linearity was evaluated over concentration ranges of 0.125–0.375 µg/mL for PST and 117.5–352.5 µg/mL for FST. Calibration curves showed excellent correlation coefficients ($R^2$ > 0.999), confirming a linear detector response.

| Concentration (µg/mL) | PST Mean Peak Area | Concentration (µg/mL) | FST Mean Peak Area |
|---|---|---|---|
| 0.125 | 167745 | 117.5 | 1099837 |
| 0.190 | 267304 | 176.25 | 1762909 |
| 0.250 | 359846 | 235.0 | 2384416 |
| 0.312 | 459109 | 293.75 | 3047378 |
| 0.375 | 551146 | 352.5 | 3973889 |

**Table 1: Linearity data for Palonosetron and Fosnetupitant.**

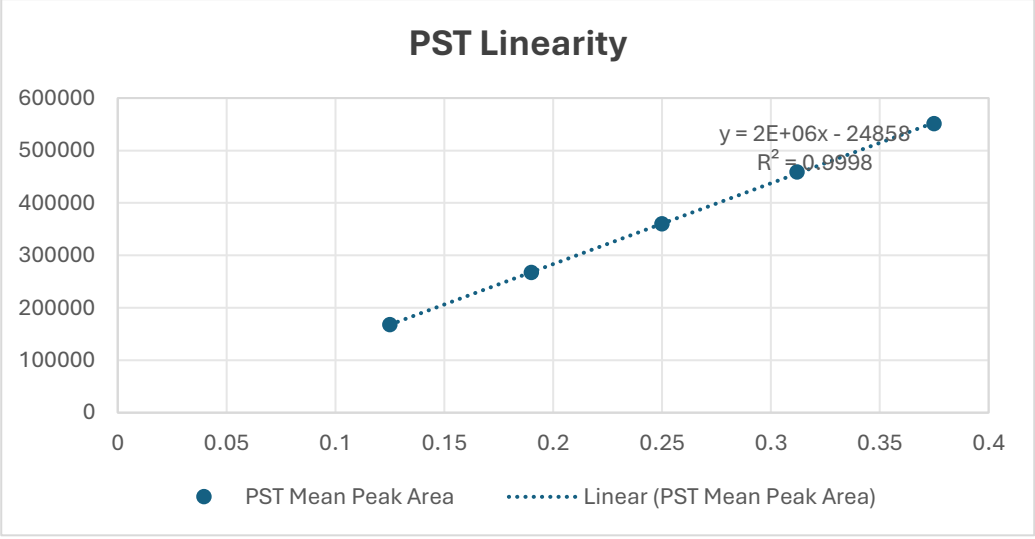

Figure No. 2 Linearity Curve for PST

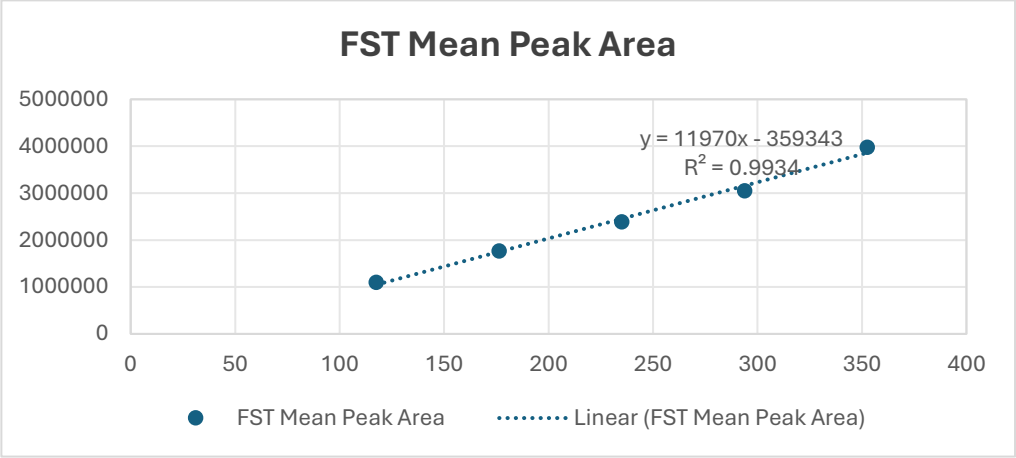


**Figure no -3 Linearity curve for FST**

These linearity results suggest that the method is suitable for a wide range of concentrations, enabling both low and high-level quantification in routine assays.

**Accuracy and Precision**

The developed method was found to be highly accurate, with recovery values ranging between 99–101% for both PST and FST. This validates that excipients in *Akynzeo I.V.* did not interfere with analyte quantification. Precision results from intra-day and inter-day analyses showed %RSD < 1%, confirming excellent reproducibility. Repeatability studies with six injections yielded consistent retention times and peak areas.

| Injection No. | PST Peak Area | FST Peak Area |
|---|---|---|
| 1 | 357450 | 2368788 |
| 2 | 356652 | 2364192 |
| 3 | 357521 | 2363600 |
| 4 | 357405 | 2362952 |
| 5 | 357158 | 2364768 |
| 6 | 356881 | 2360723 |
| Mean | 357178 | 2364171 |
| SD | 348.8 | 2659.9 |
| %RSD | 0.29 | 0.21 |

**Table 2: Precision data for Palonosetron and Fosnetupitant.**

The developed method was found to be highly accurate, with recovery values ranging between 99–101% for both PST and FST. This validates that excipients in *Akynzeo I.V.* did not interfere with analyte quantification. Precision results from intra-day and inter-day analyses showed %RSD < 1%, confirming excellent reproducibility. Repeatability studies with six injections yielded consistent retention times and peak areas.

**Sensitivity and Limit of Detection**

LOD and LOQ were calculated using the standard deviation of response and slope of calibration curves. The LOD values were 0.001 µg/mL (PST) and 0.121 µg/mL (FST), while LOQ values were 0.002 µg/mL (PST) and 0.405 µg/mL (FST). These low values indicate the method's capability to detect and quantify trace levels of both drugs, demonstrating high sensitivity suitable for trace impurity profiling.

**Accuracy and Recovery**

Accuracy was evaluated by the standard addition method at three levels (50%, 100%, and 150%). Recovery values were found between 99% and 101%, indicating good accuracy.

| Recovery Level | PST Amount Taken (µg/mL) | PST Amount Found (µg/mL) | PST Recovery (%) | FST Amount Taken (µg/mL) | FST Amount Found (µg/mL) | FST Recovery (%) |
|---|---|---|---|---|---|---|
| 50% | 0.124 | 0.12 | 99 | 115.15 | 114.96 | 100 |
| 100% | 0.248 | 0.25 | 100 | 230.30 | 231.09 | 100 |
| 150% | 0.371 | 0.37 | 100 | 345.45 | 346.76 | 100 |

**Table 3: Accuracy and recovery results of Palonosetron and Fosnetupitant.**

**Stability Studies**

Forced degradation studies demonstrated the stability-indicating capability of the method. The degradation percentage of both drugs was minimal, confirming robustness under various conditions.

| Stress Condition | PST Response Area | PST % Degraded | PST % Remaining | FST Response Area | FST % Degraded | FST % Remaining |
|---|---|---|---|---|---|---|
| 0.1 N HCl | 312743 | 12.96 | 87.04 | 2121264 | 11.04 | 88.96 |
| 0.1 N NaOH | 325222 | 9.49 | 90.51 | 2152023 | 9.75 | 90.25 |
| 30% $H_2O_2$ | 336158 | 6.45 | 93.55 | 2197100 | 7.86 | 92.14 |
| Sunlight | 335254 | 6.70 | 93.30 | 2204745 | 7.54 | 92.46 |
| 105°C | 306817 | 14.61 | 85.39 | 2044211 | 14.27 | 85.73 |

**Table 4: Stability study results of Palonosetron and Fosnetupitant.**

**Robustness and Ruggedness**

The robustness was confirmed by introducing deliberate minor changes in method parameters such as flow rate (±0.1 mL/min), detection wavelength (±2 nm), and mobile phase ratio (±5%). These changes did not significantly affect retention time, peak area, or symmetry, validating the stability of the method. Ruggedness was tested by different analysts and instruments, yielding consistent results, proving its reliability in routine laboratory applications.

**Specificity and Selectivity**

Specificity was demonstrated by comparing chromatograms of blank, placebo, standard, and sample solutions. No interfering peaks were observed at the retention times of PST and FST, confirming that excipients and diluents did not interfere. Selectivity was further confirmed by spiking known quantities of standards into the sample solution, yielding recoveries within the 98–102% range.

**Forced Degradation and Stability-Indicating Nature**

Forced degradation studies under acidic, basic, oxidative, thermal, and photolytic conditions confirmed the stability-

indicating capability of the developed method. Both PST and FST showed minor degradation under strong stress, but their main peaks were well resolved from degradation products, confirming that the method could differentiate between analytes and degradation components. Degradation followed the order: thermal > acidic > alkaline > photolytic > oxidative, consistent with the chemical nature of both drugs.

**Comparative Evaluation with Reported Methods**

Compared to previously reported HPLC methods for Palonosetron and Fosnetupitant, the present method offers advantages such as shorter analysis time, improved sensitivity, reduced solvent consumption, and enhanced peak symmetry. Most earlier methods reported longer retention times (>6 min) and higher organic solvent usage. This optimized method thus supports green analytical principles and cost-effectiveness for large-scale quality control laboratories.

**Application to Pharmaceutical Formulation**

The validated RP-HPLC method was successfully applied to the analysis of *Akynzeo I.V.* formulation. The assay results were within 98–102% of the labeled claim for both drugs, confirming its suitability for commercial batch release and routine testing. The high precision and reproducibility ensure its potential application in both formulation development and stability studies.

## CONSLUSION

A stability-indicating RP-HPLC method was successfully developed and validated for the simultaneous estimation of Palonosetron and Fosnetupitant in *Akynzeo I.V.* The method is precise, accurate, specific, and robust, with a short analysis time. It is suitable for routine quality control and stability testing in pharmaceutical industries.

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