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Research Article

DEVELOPMENT OF NEW ANALYTICAL METHOD AND VALIDATION OF ANTI-NEOPLASTIC AGENTS NETUPITANT AND PALONOSETRON IN PURE AND PHARMACEUTICAL FORMULATION BY RP-HPLC

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Abstract:

A novel, precise, accurate, rapid and cost effective isocratic reverse phase high performance liquid chromatographic (RP-HPLC) method was developed, optimized and validated for the estimation of Netupitant (NET) and Palonosetron (PAL) in bulk and pharmaceutical dosage forms. The drugs were estimated using Phenomenex Gemini C18 (4.6mm×150mm, 5µm) particle size column. A mobile phase composed of tri ethylamine buffer and methanol in proportion of 32:68 v/v, at a flow rate of 1.0 ml/min was used for the separation. Detection was carried out at 248nm. The linearity range obtained was 30-70µg/ml for Netupitant and 10-50µg/ml for Palonosetron with retention times (Rt) of 3.297min and 5.405min for Netupitant and Palonosetron respectively. The correlation coefficient values were found to be 0.999 & 0.999. Precision studies showed % RSD values less than 2 % for both the drugs in all the selected concentrations. The percentage recoveries of Netupitant (NET) and Palonosetron (PAL) were found to be 100.1873% for Netupitant and 100.748% for Palonosetron respectively. The assay results of Netupitant (NET) and Palonosetron (PAL) were found to be 99.82%. The limit of detection (LOD) and limit of quantification (LOQ) were 2.6µg/ml and 7.8µg/ml for Netupitant and 3.4µg/ml 10.2µg/ml for Palonosetron respectively. The proposed method was validated as per the International Conference on Harmonization (ICH) guidelines. The proposed validated method was successfully used for the quantitative analysis of commercially available dosage form.

Keywords: Netupitant and Palonosetron, RP-HPLC, ICH Guidelines, Validation.

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INTRODUCTION:**Chromatography****Introduction**

The chromatography was discovered by Russian Chemist and botanist *Micheal Tswett* (1872-1919) who first used the term chromatography (colour writing derived from Greek for colour – Chroma, and write – graphein) to describe his work on the separation of coloured plant pigments into bands on a column of chalk and other material such as polysaccharides, sucrose and insulin.

“Chromatography is a method in which the components of a mixture are separated on an adsorbent column in a flowing system”.

The adsorbent material, or stationary phase, first described by Russian scientist named Tswett in 1906, has taken many forms over the years, including paper, thin layers of solids attached to glass plates, immobilized liquids, gels, and solid particles packed in columns.

“Chromatography is a physical method of separation in which the component to be separated are distributed between two phases of which in stationary while other moves in a definite direction (IUPAC)”

Types of Chromatography

The mobile phase could be either a liquid or a gas, and accordingly we can subdivide chromatography into Liquid Chromatography (LC) or Gas Chromatography (GC). Apart from these methods, there are two other modes that use a liquid mobile phase, but the nature of its transport through the porous stationary phase is in the form of either (a) capillary forces, as in planar chromatography (also called Thin-Layer Chromatography, TLC), or (b) electro osmotic flow, as in the case of Capillary Electro Chromatography (CEC).

1. Adsorption chromatography

Chromatography in which separation is based mainly on difference between the adsorption affinities of the sample components for the surface of an active solid. The analyte interact with solid stationary surface and are displaced with eluent for active sites on surface.

2. Partition chromatography

This method results from a thermodynamic distribution of analytes between two liquid phases. On the basis of relative polarities of stationary and mobile phase, partition chromatography can be divided in to normal phase and reverse phase chromatography. In normal phase chromatography, the stationary phase bed is strongly polar in nature (e.g. Silica gel) and the mobile phase is non-polar (such as n-hexane or tetrahydrofuran). Polar sample are thus retained on polar surface of the column packing longer than polar

material while in reverse phase chromatography, the stationary bed is non-polar (hydrophobic in nature, while the mobile phase is polar liquid, such as mixture of water and methanol or Acetonitrile. Here the more non polar the material is, the longer it will retain.

3. Size-exclusion chromatography

This involves a solid stationary phase with controlled pore size. Solids are separated according to molecular size, with the large molecule unable to enter the pores eluted first.

4. Ion- exchange chromatography

Involves a solid stationary phase with anionic or cationic groups on the surface to separation, HPLC and HPTLC methods have widely been exploited in pharmaceutical analysis because of its simplicity, precision, accuracy and reproducibility of result.

5. Solid-Phase Extraction [SPE]

A sample preparation technique that uses LC principles to isolate, enriches, and/or purifies analytes from a complex matrix applied to a miniature chromatographic bed. *Offline* SPE is done with larger particles in individual plastic cartridges or in micro-elution plate wells, using low positive pressure or vacuum to assist flow. *Online* SPE is done with smaller particles in miniature HPLC columns using higher pressures and a valve to switch the SPE column online with the primary HPLC column, or offline to waste, as appropriate. SPE methods use step gradients to accomplish bed conditioning, sample loading, washing, and elution steps. The goal is to remove matrix interferences and to isolate the analyte in a solution, and at a concentration, suitable for subsequent analysis.

High Performance Liquid Chromatography (HPLC)

The acronym *HPLC*, coined by the Late Prof. Csaba Horvath for his 1970 Pittconpaper, originally indicated the fact that high pressure was used to generate the flow required for liquid chromatography in packed columns. In the beginning, pumps only had a pressure capability of 500 psi [35 bars]. This was called *high pressure liquid chromatography*, or HPLC. The early 1970s saw a tremendous leap in technology. These new HPLC instruments could develop up to 6,000 psi [400 bars] of pressure, and incorporated improved injectors, detectors, and columns. With continued advances in performance during this time [smaller particles, even higher pressure], the acronym HPLC remained the same, but the name was changed to high performance liquid chromatography.

High Performance Liquid Chromatography is now one of the most powerful tools in analytical chemistry. It has the ability to separate, identify, and quantitative the compounds that are present in any sample that can be dissolved in a liquid. Today, compounds in trace concentrations as low as *parts per trillion* (ppt) may easily be identified. HPLC can be, and has been, applied to just about any sample, such as pharmaceuticals, food, nutraceuticals, cosmetics, environmental matrices, forensic samples, and industrial chemicals.

MATERIALS AND METHODS:

Netupitant (Pure) & Palonosetron (Pure) Procured from Sura labs, Water and Methanol for HPLC from LICHROSOLV (MERCK), Acetonitrile for HPLC from Merck, Triethylamine from Merck.

HPLC METHOD DEVELOPMENT: TRAILS

Preparation of standard solution:

Accurately weigh and transfer 10 mg of Netupitant and Palonosetron working standard into a 10ml of clean dry volumetric flasks add about 7ml of Methanol and sonicate to dissolve and removal of air completely and make volume up to the mark with the same Methanol.

Further pipette 2.25ml of the above Netupitant and 0.45ml of the Palonosetron stock solutions into a 10ml volumetric flask and dilute up to the mark with Methanol.

Procedure:

Inject the samples by changing the chromatographic conditions and record the chromatograms, note the conditions of proper peak elution for performing validation parameters as per ICH guidelines.

Mobile Phase Optimization:

Initially the mobile phase tried was Methanol: Water, Acetonitrile: Water with varying proportions. Finally, the mobile phase was optimized to Methanol: TEA buffer pH 4.8 in proportion 32:68 v/v respectively.

Optimization of Column:

The method was performed with various columns like C18 column, X- bridge column, Xterra. Phenomenex Gemini C18 (4.6mm×150mm, 5.0 µm) particle size was found to be ideal as it gave good peak shape and resolution at 1ml/min flow.

OPTIMIZED CONDITIONS:

Instrument used : Waters HPLC with auto sampler and PDA Detector 996 model.

Column : Phenomenex Gemini C18 (4.6mm×150mm, 5.0 µm) particle size

Column temperature : 38°C

pH : 4.8

Mobile phase : Methanol: TEA buffer pH 4.8 (32:68v/v)

Flow rate : 1ml/min

Wavelength : 248nm

Injection volume : 20µl

Run time : 7 min

METHOD VALIDATION

PREPARATION OF MOBILE PHASE:

Preparation of mobile phase:

Accurately measured 320ml (32%) of HPLC Methanol and 680ml of TEA buffer (68%) were mixed and degassed in a digital ultra sonicator for 15 minutes and then filtered through 0.45 µ filter under vacuum filtration.

Diluent Preparation:

The Mobile phase was used as the diluent.

RESULTS AND DISCUSSION:

Column : Phenomenex Gemini C18 (4.6mm×150mm, 5.0 µm) particle size

Column temperature : 38°C

Wavelength : 248nm

Mobile phase ratio : Methanol: TEA buffer pH 4.8 (32:68v/v)

Flow rate : 1ml/min

Injection volume : 20µl

Run time : 7minutes

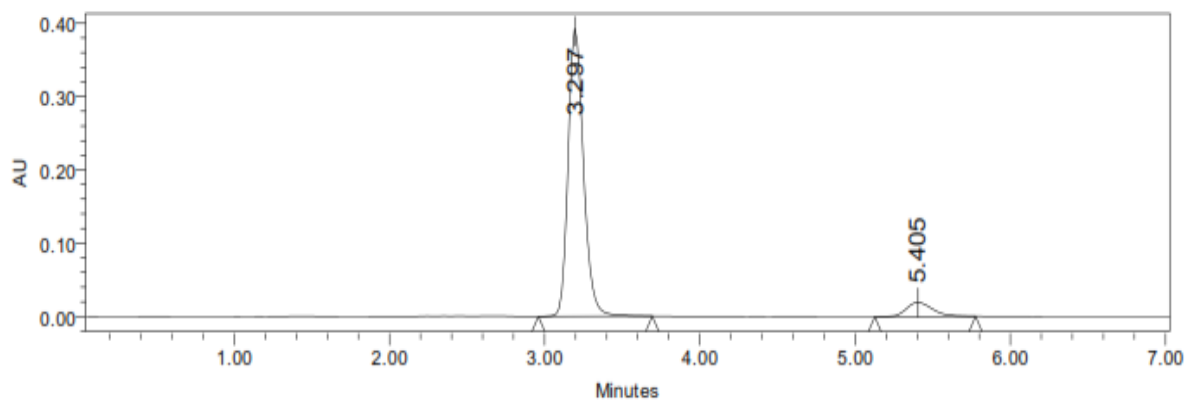


Figure-: Optimized Chromatogram (Standard)

Table-: Optimized Chromatogram (Standard)

| S.No | Name | RT | Area | Height | USP Tailing | USP Plate Count | USP Resolution |
|------|--------------|-------|--------|--------|-------------|-----------------|----------------|
| 1 | Netupitant | 3.297 | 859856 | 42569 | 1.24 | 7896 | |
| 2 | Palonosetron | 5.405 | 5698 | 3652 | 1.36 | 6582 | 6.8 |

Observation: From the above chromatogram it was observed that the Netupitant and Palonosetron peaks are well separated and they shows proper retention time, resolution, peak tail and plate count. So it's optimized trial.

Optimized Chromatogram (Sample)

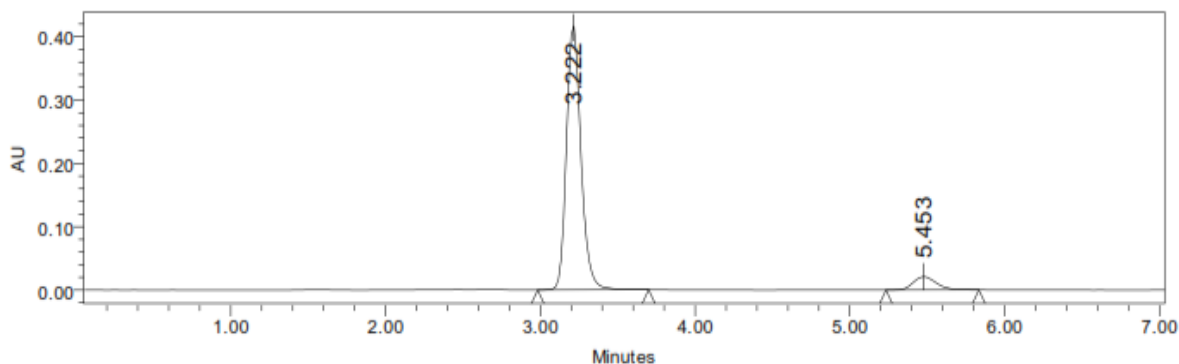


Figure-: Optimized Chromatogram (Sample)

Table-: Optimized Chromatogram (Sample)

| S.No | Name | RT | Area | Height | USP Tailing | USP Plate Count | USP Resolution |
|------|--------------|-------|--------|--------|-------------|-----------------|----------------|
| 1 | Netupitant | 3.222 | 865898 | 43659 | 1.26 | 7985 | |
| 2 | Palonosetron | 5.453 | 5789 | 3785 | 1.38 | 6659 | 7.0 |

Table-: Results of system Suitability for Netupitant

| S.No. | Peak Name | RT | Area ($\mu\text{V} \cdot \text{sec}$) | Height (μV) | USP Plate Count | USP Tailing |
|------------------|------------|-------|---|--------------------------|-----------------|-------------|
| 1 | Netupitant | 3.200 | 859865 | 42568 | 7895 | 1.24 |
| 2 | Netupitant | 3.248 | 859788 | 42587 | 7859 | 1.24 |
| 3 | Netupitant | 3.299 | 857984 | 42659 | 7869 | 1.24 |
| 4 | Netupitant | 3.297 | 854879 | 42875 | 7849 | 1.24 |
| 5 | Netupitant | 3.297 | 857896 | 42487 | 7859 | 1.23 |
| Mean | | | 858082.4 | | | |
| Std. Dev. | | | 2024.409 | | | |
| % RSD | | | 0.235922 | | | |

Acceptance Criteria:

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

Table-: Results of System Suitability for Palonosetron

| S.No | Peak Name | RT | Area ($\mu\text{V} \cdot \text{sec}$) | Height (μV) | USP Plate Count | USP Tailing |
|------------------|--------------|-------|---|--------------------------|-----------------|-------------|
| 1 | Palonosetron | 5.413 | 5689 | 3659 | 6583 | 1.36 |
| 2 | Palonosetron | 5.484 | 5687 | 3648 | 6592 | 1.37 |
| 3 | Palonosetron | 5.405 | 5682 | 3698 | 6549 | 1.37 |
| 4 | Palonosetron | 5.405 | 5649 | 3675 | 6571 | 1.36 |
| 5 | Palonosetron | 5.409 | 5674 | 3649 | 6529 | 1.36 |
| Mean | | | 5676.2 | | | |
| Std. Dev. | | | 16.2696 | | | |
| % RSD | | | 0.286628 | | | |

Acceptance Criteria:

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

Assay (Standard):**Table-: Peak Results for Assay Standard****Netupitant**

| S.No. | Name | RT | Area | Height | USP Tailing | USP Plate Count |
|-------|------------|-------|--------|--------|-------------|-----------------|
| 1 | Netupitant | 3.211 | 859785 | 42598 | 1.25 | 7856 |
| 2 | Netupitant | 3.222 | 859865 | 42895 | 1.24 | 7859 |
| 3 | Netupitant | 3.254 | 857849 | 42578 | 1.25 | 7869 |

Palonosetron

| S.No | Name | RT | Area | Height | USP Tailing | USP Plate Count | Resolution |
|------|--------------|-------|------|--------|-------------|-----------------|------------|
| 1 | Palonosetron | 5.414 | 5699 | 3685 | 1.36 | 6598 | 6.9 |
| 2 | Palonosetron | 5.453 | 5687 | 3659 | 1.37 | 6537 | 6.9 |
| 3 | Palonosetron | 5.424 | 5689 | 3649 | 1.36 | 6582 | 7.0 |

Assay (Sample):**Table-: Peak Results for Assay sample****Netupitant**

| S.No | Name | RT | Area | Height | USP Tailing | USP Plate Count |
|------|------------|-------|--------|--------|-------------|-----------------|
| 1 | Netupitant | 3.297 | 865985 | 43659 | 1.26 | 7985 |
| 2 | Netupitant | 3.294 | 865798 | 43875 | 1.26 | 7925 |
| 3 | Netupitant | 3.295 | 865456 | 43659 | 1.27 | 7946 |

Palonosetron

| S.No | Name | RT | Area | Height | USP Tailing | USP Plate Count | Resolution |
|------|--------------|-------|------|--------|-------------|-----------------|------------|
| 1 | Palonosetron | 5.435 | 5789 | 3659 | 1.37 | 6659 | 6.9 |
| 2 | Palonosetron | 5.417 | 5798 | 3684 | 1.38 | 6689 | 7.0 |
| 3 | Palonosetron | 5.434 | 5749 | 3695 | 1.38 | 6648 | 6.9 |

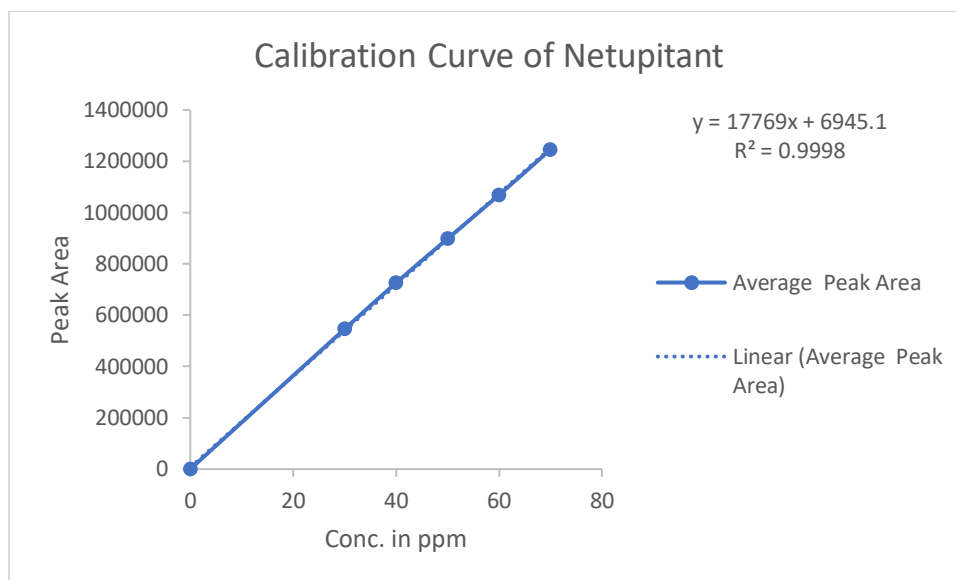
%ASSAY =

$$\frac{\text{Sample area}}{\text{Standard area}} \times \frac{\text{Weight of standard}}{\text{Dilution of standard}} \times \frac{\text{Dilution of sample}}{\text{Weight of sample}} \times \frac{\text{Purity}}{100} \times \frac{\text{Weight of tablet}}{\text{Label claim}} \times 100$$

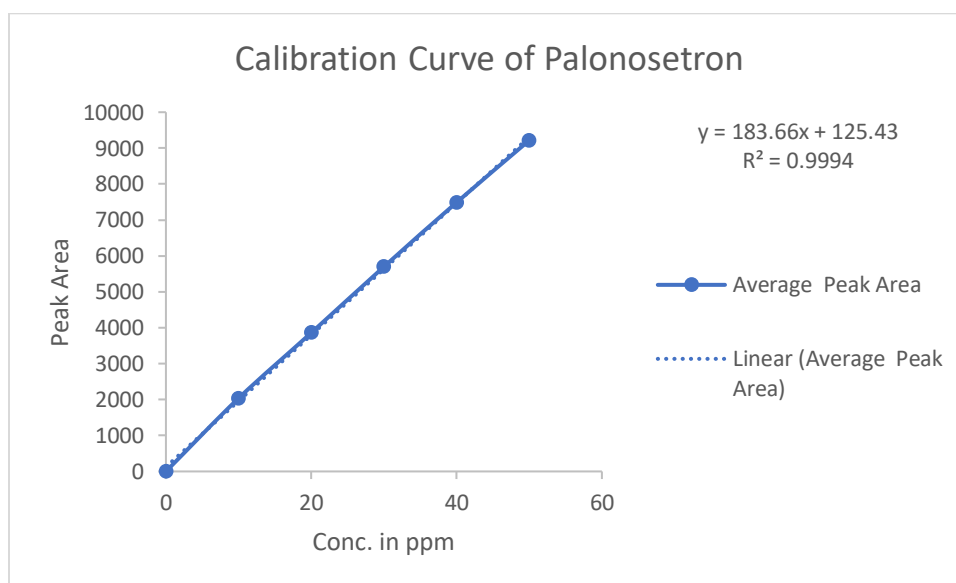
The % purity of Netupitant and Palonosetron in pharmaceutical dosage form was found to be 99.82%.

LINEARITY**Netupitant**

| Concentration µg/ml | Average Peak Area |
|------------------------|----------------------|
| 30 | 545894 |
| 40 | 725985 |
| 50 | 897856 |
| 60 | 1068594 |
| 70 | 1245698 |

**Fig-: Calibration Curve of Netupitant****Palonosetron**

| Concentration μg/ml | Average Peak Area |
|------------------------|----------------------|
| 10 | 2038 |
| 20 | 3859 |
| 30 | 5698 |
| 40 | 7489 |
| 50 | 9218 |

**Fig-: Calibration Curve of Palonosetron**

REPEATABILITY**Table-: Results of Repeatability for Netupitant:**

| S. No. | Peak name | Retention time | Area($\mu\text{V}\cdot\text{sec}$) | Height (μV) | USP Plate Count | USP Tailing |
|----------------|------------|----------------|--------------------------------------|--------------------------|-----------------|-------------|
| 1 | Netupitant | 3.213 | 859856 | 42659 | 7859 | 1.24 |
| 2 | Netupitant | 3.253 | 857985 | 42598 | 7869 | 1.24 |
| 3 | Netupitant | 3.297 | 856984 | 42587 | 7846 | 1.25 |
| 4 | Netupitant | 3.215 | 856987 | 42569 | 7819 | 1.25 |
| 5 | Netupitant | 3.254 | 859878 | 42894 | 7856 | 1.24 |
| Mean | | | 858338 | | | |
| Std.dev | | | 1454.222 | | | |
| %RSD | | | 0.169423 | | | |

Acceptance criteria:

- %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.

Table-: Results of repeatability for Palonosetron:

| S. No. | Peak Name | Retention time | Area($\mu\text{V}\cdot\text{sec}$) | Height (μV) | USP Plate Count | USP Tailing |
|----------------|--------------|----------------|--------------------------------------|--------------------------|-----------------|-------------|
| 1 | Palonosetron | 5.441 | 5697 | 3659 | 6592 | 1.36 |
| 2 | Palonosetron | 5.442 | 5689 | 3648 | 6539 | 1.36 |
| 3 | Palonosetron | 5.409 | 5698 | 3692 | 6584 | 1.37 |
| 4 | Palonosetron | 5.520 | 5639 | 3648 | 6579 | 1.36 |
| 5 | Palonosetron | 5.424 | 5688 | 3689 | 6549 | 1.36 |
| Mean | | | 5682.2 | | | |
| Std.dev | | | 24.57031 | | | |
| %RSD | | | 0.432408 | | | |

Intermediate precision:**Table-: Results of Intermediate precision for Netupitant**

| S.No. | Peak Name | RT | Area ($\mu\text{V}\cdot\text{sec}$) | Height (μV) | USP Plate count | USP Tailing |
|------------------|------------|-------|---------------------------------------|--------------------------|-----------------|-------------|
| 1 | Netupitant | 3.211 | 868956 | 43659 | 7985 | 1.26 |
| 2 | Netupitant | 3.211 | 869857 | 43985 | 7954 | 1.27 |
| 3 | Netupitant | 3.210 | 865983 | 43879 | 7946 | 1.26 |
| 4 | Netupitant | 3.212 | 866587 | 43865 | 7963 | 1.27 |
| 5 | Netupitant | 3.211 | 864256 | 43875 | 7964 | 1.26 |
| 6 | Netupitant | 3.297 | 868974 | 43562 | 7942 | 1.26 |
| Mean | | | 867435.5 | | | |
| Std. Dev. | | | 2167.095 | | | |
| % RSD | | | 0.249828 | | | |

Acceptance criteria:

- %RSD of six different sample solutions should not more than 2.

Table-: Results of Intermediate precision for Palonosetron

| S.No. | Peak Name | RT | Area ($\mu\text{V} \cdot \text{sec}$) | Height (μV) | USP Plate count | USP Tailing |
|------------------|--------------|-------|---|--------------------------|-----------------|-------------|
| 1 | Palonosetron | 5.411 | 5785 | 3789 | 6659 | 1.37 |
| 2 | Palonosetron | 5.410 | 5798 | 3758 | 6625 | 1.38 |
| 3 | Palonosetron | 5.420 | 5766 | 3746 | 6649 | 1.38 |
| 4 | Palonosetron | 5.423 | 5746 | 3795 | 6675 | 1.37 |
| 5 | Palonosetron | 5.419 | 5782 | 3761 | 6653 | 1.38 |
| 6 | Palonosetron | 5.409 | 5786 | 3752 | 6627 | 1.37 |
| Mean | | | 5777.167 | | | |
| Std. Dev. | | | 18.40018 | | | |
| % RSD | | | 0.318498 | | | |

Acceptance Criteria:

- %RSD of six different sample solutions should not more than 2.

Table-: Results of Intermediate precision Day 2 for Netupitant

| S.No. | Peak Name | RT | Area ($\mu\text{V} \cdot \text{sec}$) | Height (μV) | USP Plate Count | USP Tailing |
|------------------|------------|-------|---|--------------------------|-----------------|-------------|
| 1 | Netupitant | 3.211 | 845985 | 44585 | 8025 | 1.27 |
| 2 | Netupitant | 3.233 | 847895 | 44895 | 8069 | 1.28 |
| 3 | Netupitant | 3.244 | 848985 | 44758 | 8046 | 1.27 |
| 4 | Netupitant | 3.297 | 847859 | 44548 | 8094 | 1.28 |
| 5 | Netupitant | 3.297 | 845984 | 44865 | 8042 | 1.28 |
| 6 | Netupitant | 3.202 | 847898 | 44254 | 8076 | 1.27 |
| Mean | | | 847434.3 | | | |
| Std. Dev. | | | 1201.345 | | | |
| % RSD | | | 0.141763 | | | |

Acceptance Criteria:

- %RSD of six different sample solutions should not more than 2.

Table-: Results of Intermediate precision Day 2 for Palonosetron

| S.No. | Peak Name | RT | Area ($\mu\text{V}\cdot\text{sec}$) | Height (μV) | USP Plate Count | USP Tailing |
|------------------|--------------|-------|---------------------------------------|--------------------------|-----------------|-------------|
| 1 | Palonosetron | 5.411 | 5898 | 3986 | 6852 | 1.39 |
| 2 | Palonosetron | 5.410 | 5884 | 3955 | 6864 | 1.39 |
| 3 | Palonosetron | 5.420 | 5863 | 3956 | 6829 | 1.40 |
| 4 | Palonosetron | 5.405 | 5845 | 3945 | 6874 | 1.39 |
| 5 | Palonosetron | 5.409 | 5896 | 3925 | 6829 | 1.39 |
| 6 | Palonosetron | 5.463 | 5874 | 3962 | 6825 | 1.40 |
| Mean | | | 5876.667 | | | |
| Std. Dev. | | | 20.39281 | | | |
| % RSD | | | 0.347013 | | | |

Acceptance Criteria:

- %RSD of six different sample solutions should not more than 2.

ACCURACY:**Table-: The accuracy results for Netupitant**

| %Concentration (at specification Level) | Area | Amount Added (ppm) | Amount Found (ppm) | % Recovery | Mean Recovery |
|---|----------|--------------------|--------------------|------------|---------------|
| 50% | 451144.3 | 25 | 24.998 | 99.992% | 100.1873% |
| 100% | 897248.3 | 50 | 50.104 | 100.208% | |
| 150% | 1344562 | 75 | 75.278 | 100.362% | |

Acceptance Criteria:

- The percentage recovery was found to be within the limit (98-102%).

The results obtained for recovery at 50%, 100%, 150% are within the limits. Hence method is accurate.

Table-: The accuracy Results for Palonosetron

| %Concentration (at specification Level) | Area | Amount Added (ppm) | Amount Found (ppm) | % Recovery | Mean Recovery |
|---|----------|--------------------|--------------------|------------|---------------|
| 50% | 2895 | 15 | 15.084 | 100.560% | 100.748% |
| 100% | 5685.333 | 30 | 30.282 | 100.940% | |
| 150% | 8449 | 45 | 45.335 | 100.744% | |

Acceptance Criteria:

- The percentage recovery was found to be within the limit (98-102%).

The results obtained for recovery at 50%, 100%, 150% are within the limits. Hence method is accurate.

Robustness**Netupitant**

| Parameter used for sample analysis | Peak Area | Retention Time | Theoretical plates | Tailing factor |
|---|-----------|----------------|--------------------|----------------|
| Actual Flow rate of 1.0mL/min | 859856 | 3.297 | 7896 | 1.24 |
| Less Flow rate of 0.9mL/min | 915847 | 3.639 | 7251 | 1.20 |
| More Flow rate of 1.1mL/min | 842564 | 2.859 | 7415 | 1.21 |
| Less organic phase (about 5 % decrease in organic phase) | 825498 | 3.460 | 7365 | 1.23 |
| More organic phase (about 5 % Increase in organic phase) | 814578 | 3.022 | 7258 | 1.22 |

Acceptance Criteria:

The tailing factor should be less than 2.0 and the number of theoretical plates (N) should be more than 2000.

Table-: Results for Robustness

| Parameter used for sample analysis | Peak Area | Palonosetron Retention Time | Theoretical plates | Tailing factor |
|---|-----------|-----------------------------|--------------------|----------------|
| Actual Flow rate of 1.1mL/min | 5698 | 5.405 | 6582 | 1.36 |
| Less Flow rate of 0.9mL/min | 6452 | 6.250 | 6785 | 1.32 |
| More Flow rate of 0.8mL/min | 5254 | 4.863 | 6365 | 1.34 |
| Less organic phase (about 5 % decrease in organic phase) | 5487 | 6.196 | 6254 | 1.38 |
| More organic phase (about 5 % Increase in organic phase) | 5369 | 5.010 | 6298 | 1.33 |

Acceptance Criteria:

The tailing factor should be less than 2.0 and the number of theoretical plates (N) should be more than 2000.

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 TEA buffer was p^H 4.8 and the mobile phase was optimized with consists of Methanol: TEA buffer mixed in the ratio of 32:68 % v/v.

A Phenomenex Gemini C18 (4.6mm×150mm, 5.0 μ m) particle size or equivalent chemically bonded to porous silica particles was used as stationary phase. The solutions were chromatographed at a constant flow rate of 1.0 ml/min. The linearity range of Netupitant and Palonosetron were found to be from 30-70 μ g/ml, 10-50 μ g/ml respectively. Linear regression coefficient was not more than 0.999, 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 Netupitant and Palonosetron. LOD and LOQ were found to be within limit.

The results obtained on the validation parameters met ICH and USP requirements. It inferred the method found to be simple, accurate, precise and linear.

The method was found to be having suitable application in routine laboratory analysis with high degree of accuracy and precision.

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