*IOSR Journal Of Pharmacy And Biological Sciences (IOSR-JPBS)*
*e-ISSN:2278-3008, p-ISSN:2319-7676. Volume 16, Issue 6 Ser. III (Nov. –Dec. 2021), PP 30-34*
*www.Iosrjournals.Org*

# Method Development and Validation for the Simultaneous Estimation of Tianeptine in Bulk and Pharmaceutical Dosage Forms by RP-HPLC Method.

Malichetla Padma[1], Dr. P.V. MADHAVI LATHA[2]
*#1 Master of Pharmacy Scholar, Department of Pharmaceutical Analysis,*
*#2 Professor, Department of Pharmaceutical Analysis, Viswanadha Institute of Pharmaceutical Sciences, Visakhapatnam, AP, India.*

***Abstract***
*A rapid and precise Reverse Phase High Performance Liquid Chromatographic method has been developed for the validated of Tianeptine, in its pure form as well as in tablet dosage form. Chromatography was carried out on a Zorbax C18 (4.6 x 250mm, 5µm) column using a mixture of Water and Methanol (85:15% v/v) as the mobile phase at a flow rate of 1.0ml/min, the detection was carried out at 218nm. The retention time of the Tianeptine was 5.430 ±0.02min respectively. The method produce linear responses in the concentration range of 10-50mg/ml of Tianeptine. The method precision for the determination of assay was below 2.0%RSD. The method is useful in the quality control of bulk and pharmaceutical formulations.*

Date of Submission: 13-12-2021 Date of Acceptance: 28-12-2021

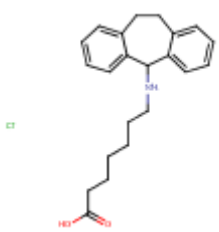
## I. Literature Review

**Malli Babu et al(2014)** The Estimation of Taineptine in tablet dosage forms by RP-HPLC, A simple, precise, rapid and accurate reverse phase HPLC method was developed for the estimation of Taineptine in tablet dosage form. An Inertsil ODS-3V analytical column (250 x 4.6 mm, 5 µm partical size) with mobile phase consisting of mixture of buffer 0.1% ortho-phosphoric acid in water and acetonitrile in the gradient program was used. The flow rate was 1.0 mL/min and the effluents were monitored at 220 nm. The retention time was 8.6 min. The detector response was linear in the concentration of 1-12 mcg/mL. The respective linear regression equation being y=1025.6x-1028.4. The limit of detection and limit of quantification was 0.005mcg /mL and 0.015mcg/mL respectively. The percentage assay of Taineptine was 99.6 %. The method was validated by determining its accuracy, precision and linearity. The results of the study showed that the proposed RP-HPLC method is simple, rapid, precise and accurate, which is useful for the routine determination of Taineptine in bulk drug and in its pharmaceutical tablet dosage form.

## II. Drug Profile:

**TIANEPTINE**

| | | |
|---|---|---|
| **Chemical name/ Nomenclature / IUPAC Name** | **:** | N-(6-carboxyhexyl)tricyclo[9.4.0.0$^{3}$,$^{8}$]pentadeca-1(15),3,5,7,11,13-hexaen-2-aminium chloride. |
| **Molecular Formula** | **:** | $C_{21}H_{25}ClN_2O_4S$ |
| **Molecular Weight** | **:** | 436.953 g/mol |
| **Solubility** | **:** | Soluble in water, DMSO |



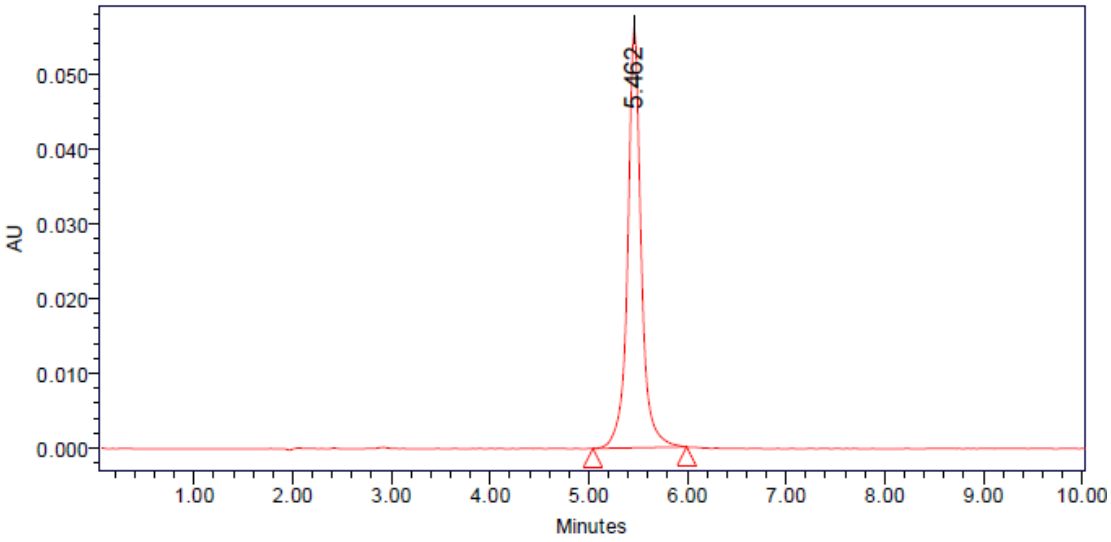
## III. Results & Discussion:

**CHROMATOGRAPHIC CONDITIONS**:

Instrument used : Waters HPLC with auto sampler and PDA detector 996 model.
Mobile phase ratio : Water: Methanol (85:15% v/v)
Column : Zorbax C18 (4.6×250mm) 5µ
Column temperature : 35ºC
Wavelength : 218nm
Flow rate : 1ml/min
Injection volume : 10µl
Run time : 10min

| S.No | Name | RT | Area | Height | USP Tailing | USP Plate Count |
|---|---|---|---|---|---|---|
| 1 | Tianeptine | 5.430 | 530023 | 56127 | 1.03 | 9118 |

it shows proper peak, tailing, platecount and baseline in the chromatogram. So it's optimized chromatogram




**SYSTEM SUITABILITY**

| S.No | Peak Name | RT | Area (µV*sec) | Height (µV) | USP Plate Count | USP Tailing |
|---|---|---|---|---|---|---|
| 1 | Tianeptine | 5.474 | 507837 | 54219 | 8931.7 | 1.1 |
| 2 | Tianeptine | 5.466 | 503577 | 56095 | 92356 | 1.0 |
| 3 | Tianeptine | 5.474 | 507837 | 54219 | 8931.7 | 1.1 |
| 4 | Tianeptine | 5.452 | 522826 | 55808 | 9070 | 1.0 |
| 5 | Tianeptine | 5.446 | 519895 | 55577 | 8987 | 1.0 |
| **Mean** | | | 512394.4 | | | |
| **Std. Dev.** | | | 8431.542 | | | |
| **% RSD** | | | 1.64 | | | |

3

**SPECIFICITY**

specificity as the ability to assess unequivocally the analyte in the presence of components that may be expected to be present, such as impurities, degradation products, and matrix components.

Analytical method was tested for specificity to measure accurately quantitate Tianeptine in drug product.

**Peak results for assay standard**

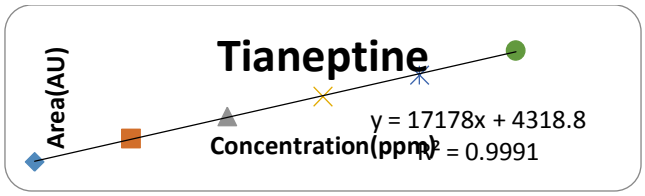
| S.No | Name | RT | Area | Height | USP Tailing | USP Plate Count | Injection |
|---|---|---|---|---|---|---|---|
| 1 | Tianeptine | 5.427 | 530023 | 56127 | 1.03 | 9118 | 1 |
| 2 | Tianeptine | 5.430 | 531649 | 56299 | 1.05 | 9364 | 2 |
| 3 | Tianeptine | 5.443 | 533969 | 55991 | 1.05 | 9186 | 3 |

Peak results for Assay sample

| S.No | Name | RT | Area | Height | USP Tailing | USP Plate Count | Injection |
|---|---|---|---|---|---|---|---|
| 1 | Tianeptine | 5.453 | 534995 | 55722 | 1.05 | 9124 | 1 |
| 2 | Tianeptine | 5.462 | 532954 | 56050 | 1.03 | 9207 | 2 |
| 3 | Tianeptine | 5.466 | 533577 | 56095 | 1.03 | 9235 | 3 |

**LINEARITY** Correlation Coefficient (r) = 0.99

| Concentration Level (%) | Concentration µg/ml | Average Peak Area |
|---|---|---|
| 33 | 10 | 182423 |
| 66 | 20 | 356108 |
| 100 | 30 | 511715 |
| 133 | 40 | 678851 |
| 166 | 50 | 873452 |




**Precision:** Obtained Five (5) replicates of 100% accuracy solution as per experimental conditions. Recorded the peak areas and calculated % RSD.

| S. No | Peak name | Retention time | Area(µV*sec) | Height (µV) | USP Plate Count | USP Tailing |
|---|---|---|---|---|---|---|
| 1 | Tianeptine | 5.419 | 507837 | 54219 | 8931.7 | 1.1 |
| 2 | Tianeptine | 5.405 | 510468 | 54508 | 8957.7 | 1.1 |
| 3 | Tianeptine | 5.478 | 514561 | 55259 | 8764.6 | 1.1 |
| 4 | Tianeptine | 5.466 | 515381 | 55552 | 9037.7 | 1.1 |
| 5 | Tianeptine | 5.466 | 516416 | 55653 | 8972.4 | 1.1 |
| **Mean** | | | 512932.6 | | | |
| **Std.dev** | | | 3633.862 | | | |
| **%RSD** | | | 0.7 | | | |

**Intermediate precision:**

| S.No | Peak Name | RT | Area (µV*sec) | Height (µV) | USP Plate Count | USP Tailing |
|---|---|---|---|---|---|---|
| 1 | Tianeptine | 5.484 | 516091 | 54804 | 9090 | 1.1 |
| 2 | Tianeptine | 5.493 | 518221 | 54903 | 9131 | 1.1 |
| 3 | Tianeptine | 5.406 | 519536 | 55996 | 9071 | 1.0 |
| 4 | Tianeptine | 5.419 | 519881 | 56102 | 9015 | 1.1 |
| 5 | Tianeptine | 5.446 | 519895 | 55577 | 8987 | 1.0 |
| 6 | Tianeptine | 5.452 | 522826 | 55808 | 9070 | 1.0 |
| **Mean** | | | 519408.3 | | | |
| **Std. Dev.** | | | 2216.82 | | | |
| **% RSD** | | | 0.42 | | | |

**ACCURACY:**

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

| %Concentration (at specification Level) | Area | Amount Added (ppm) | Amount Found (ppm) | % Recovery | Mean Recovery |
|---|---|---|---|---|---|
| 50% | 80848 | 15 | 14.74 | 98.2% | 99.3% |
| 100% | 146118 | 30 | 29.86 | 99.5% | |
| 150% | 212196.3 | 45 | 45.16 | 100.3% | |

**LIMIT OF DETECTION FOR TIANEPTINE**

The detection limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be detected but not necessarily quantitated as an exact value.

**LOD= 3.3 × σ / s**

Where

σ = Standard deviation of the response

S = Slope of the calibration curve

**Result:** =3.3×11739.84501/17178

=2.25µg/ml

**Quantitation limit**

The quantitation limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be quantitatively determined.

**LOQ=10×σ/S**

Where

σ = Standard deviation of the response

S = Slope of the calibration curve

**Result:** =10×11739.84501/17178

=6.8µg/ml

**Robustness**

The robustness was performed for the flow rate variations from 0.9 ml/min to 1.1ml/min and mobile phase ratio variation from more organic phase to less organic phase ratio for Tianeptine. The method is robust only in less flow condition and the method is robust even by change in the Mobile phase ±10%. The standard and samples of Tianeptine 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.

| Parameter used for sample analysis | Peak Area | Retention Time | Theoretical plates | Tailing factor |
|---|---|---|---|---|
| Actual Flow rate of 1.0 mL/min | 534995 | 5.453 | 9124 | 1.01 |
| Less Flow rate of 0.9 mL/min | 566441 | 5.599 | 9364 | 1.02 |
| More Flow rate of 1.1 mL/min | 459187 | 4.576 | 7559 | 0.98 |
| More Organic phase | 93382 | 3.827 | 6274 | 1.07 |
| Less organic phase | 24366 | 7.415 | 12009 | 1.00 |

## IV. Summary:

The analytical method was developed by studying different parameters. First of all, maximum absorbance was found to be at 218 nm and the peak purity was excellent. Injection volume was selected to be 10µl which gave a good peak area. The column used for study was Zorbax $C_{18}$ because it was giving good peak.35ºC temperature was found to be suitable for the nature of drug solution. The flow rate was fixed at 1.0ml/min because of good peak area and satisfactory retention time. Mobile phase is Water: Methanol (85:15% v/v) was fixed due to good symmetrical peak. So this mobile phase was used for the proposed study. Water: Methanol was selected because of maximum extraction sonication time was fixed to be 10min at which all the drug particles were completely soluble and showed good recovery. Run time was selected to be 10 min because analyze gave peak around 5.4 and also to reduce the total run time. The present recovery was found to be 98.0-102 was linear and precise over the same range. Both system and method precision was found to be accurate and well within range. The analytical method was found linearity over the range of 10-50ppm of the target concentration. The analytical passed both robustness and ruggedness tests. On both cases, relative standard deviation was well satisfactory.

## V. Conclusion :

The proposed HPLC method was found to be precise, specific, accurate, rapid and economical for simultaneous estimation of Tianeptine in tablet dosage form. The sample recoveries in all formulations were in good agreement with their respective label claims and this method can be used for routine analysis. It can be applied for routine analysis in laboratories. Tianeptine was freely soluble in acetonitrile ethanol, methanol and sparingly soluble in water. Water: Methanol (85:15% v/v) was chosen as the mobile phase. The solvent system used in this method was economical. The %RSD values were within 2 and the method was found to be precise.

## References

[1]. Dr. Kealey and P.J Haines, Analytical Chemistry, 1stedition, Bios Publisher, (2002), PP 1-7.
[2]. A.BraithWait and F.J.Smith, Chromatographic Methods, 5thedition, Kluwer Academic Publisher, (1996), PP 1-2.
[3]. Andrea Weston and Phyllisr. Brown, HPLC Principle and Practice, 1st edition, Academic press, (1997), PP 24-37.
[4]. Yuri Kazakevich and Rosario Lobrutto, HPLC for Pharmaceutical Scientists, 1stedition, Wiley Interscience A JohnWiley & Sons, Inc., Publication, (2007), PP 15-23.
[5]. Chromatography, (online). URL:http://en.wikipedia.org/wiki/Chromatography.
[6]. Meyer V.R. Practical High-Performance Liquid Chromatography, 4th Ed. England, John Wiley & Sons Ltd, (2004), PP 7-8.
[7]. Sahajwalla CG a new drug development, vol 141, Marcel Dekker Inc., New York, (2004), PP 421–426.
[8]. Introduction to Column. (Online),URL:http://amitpatel745.topcities.com/index_files/study/column care.pdf
[9]. Detectors used in HPLC (online )URL:http://wiki.answers.com/Q/What_detectors_are_used_in_HPLC
[10]. Detectors (online) ,URL:http://hplc.chem.shu.edu/NEW/HPLC_Book/Detectors/det_uvda.html
[11]. Detectors (online) ,URL:http://www.dionex.com/enus/webdocs/64842-31644-02_PDA-100.pdf

Malichetla Padma, et. al. "Method Development and Validation for the Simultaneous Estimation of Tianeptine in Bulk and Pharmaceutical Dosage Forms by RP-HPLC Method." *IOSR Journal of Pharmacy and Biological Sciences (IOSR-JPBS)*, 16(6), (2021): pp. 30-34.