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Research Article

# RP-HPLC Method Development And Validation Of Tetrabenazine With Its Known And Unknown Degradation Impurities In Its Tablet Dosage Form

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**Abstract**

Force degradation also known as Stress testing. It's process i.e. involves degradation of drug product and drug substance at condition more than accelerated condition & thus generates degradation products i.e. can be studied to determine the stability of the molecule. In the present study gradient RP-HPLC method was development for separation of impurities and degradation product from drug product. The chromatographic separation was performed on Inertsil ODS 3V (250 mm x 4.6 mm, 5 µm) using gradient elution. Other HPLC parameter which were optimised flow rate 1.0 ml/min; detection wavelength 224 nm; column oven temperature 25ºC; and injection volume 20 µl. The development method was statistically validated for linearity (1.2-6.0ppm). The Forced degradation study revealed that drug was sensitive to Peroxide degradation, in other condition not adequate degradation observed. Result of precision (% RSD < 5), accuracy, specificity are well within the limits. % Recovery at 50%, 100%, 150% was found to be within limits 70-130%.

**Keywords**: Tetrabenazine, RP-HPLC, Forced degradation, Related substances, Development & Validation.

## INTRODUCTION[1-3]

Tetrabenazine is chemically 9,10-dimethoxy-3-(2-methylpropyl)-1H,2H,3H,4H,6H,7H,11bH-pyrido[2,1-a]isoquinolin-2-one. Tetrabenazineis used in the prevention and treatment of Huntington Disease. Tetrabenazine is a reversible human vesicular monoamine transporter type 2 inhibitor. This drug acts within the basal ganglia and promotes depletion of monoamine neurotransmitters serotonin, norepinephrine, and dopamine from stores.Methods such as HPLC, LC-MS, UV-spectrophotometric, Colorimetric, and FT-IR method are reported for estimation of Tetrabenazine.A literature search reveals that few analytical methods were reported for Stability indicating RP-HPLC method development and Validation for estimation of Tetrabenazine.However, there was no analytical method reported for degradation product of Tetrabenazine by HPLC method. Hence a Simple, Rapid, Sensitive and Accurate stability indicating RP-HPLC method was developed for determination of Tetrabenazine and its related substance in its Tablet dosage form.

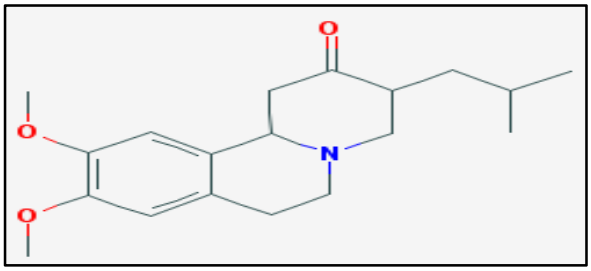


**Figure1 Tetrabenazine structure**

## MATERIALS AND METHODS[4-20]

### Material and Reagents

Acetonitrile, di Potassium Hydrogen Phosphate, di Ammonium hydrogen phosphate, Ammonium acetate, Ortho Phosphosphoric Acid, Hydrochloric acid, Sodium hydroxide, Hydrogen

peroxidewere procured from Merck and Finar Reagent. Tetrabenazine standard Gifted by ZY-DUS CADILA HEALTH CARE, Moraiya, Ahmedabad.

**Equipment**

Chromatographic separation was performed on RP-HPLC system consist of model Shimdzu LC-2010C 3D series PDA detector with 10µl loop volume. UV spectrophotometer which consists of model Shimadzu UV 1800 Spectrophotometer is also used to measure the wavelength of Tetrabenazine.

**Preparation solutions**

**Preparation of Standard Stock Solution**

Weighed accurately about 100 mg Tetrabenazine Working Standard in to a 50 ml volumetric flask add 35 ml of Diluent (ACN : Water 80:20 %v/v), sonicate to dissolve and make up volume with Diluent and mix. Take 5 ml of above solution transferred into 50 ml vol. flask

**Preparation of standard solution**

Pipette and transfer 2 ml of standard stock solution into 100 ml volumetric flask.Make volume uo to the mark with Diluent and mix.

**Preparation of Impurity Solutions**

(2-Acetyl-4-methylpentyl)trimethyl ammonium iodide(Imp-1): weighed 20 mg of trimethyl ammonium iodide into 100 ml volumetric flask. Add 5 ml ACN Sonicated to dissolve and make up volume with diluents.

2-(3,4-Dimethoxyphenyl)ethylamine(Imp-2): weighed 20 mg of 2-(3,4-Dimethoxyphenyl)ethylamine into 100 ml volumetric flask. Add 5 ml ACN Sonicated to dissolve and make up volume with diluents.

N-[2-(3,4-dimethoxyphenyl)ethyl]formamide(Imp-3): weighed 20 mg of N-[2-(3,4-dimethoxyphenyl)ethyl]formamide into 100 ml volumetric flask. Add 5 ml ACN Sonicated to dissolve and make up volume with diluents.

3,4-dihydro-6,7-dimethoxyisoquinoline(Imp-4): weighed 20 mg of 3,4-dihydro-6,7-dimethoxyisoquinoline into 100 ml volumetric flask. Add 5 ml ACN Sonicated to dissolve and make up volume with diluents.

3-Isobutyl-9,10-dimethoxy-2,3,4,6,7,11b-hexahydro-1H-benzo[a]quinolizin-2-ol(Imp-5): weighed 20 mg of 3-Isobutyl-9,10-dimethoxy-2,3,4,6,7,11b-hexahydro-1H-benzo[a]quinolizin-2-ol into 100 ml volumetric flask. Add 5 ml ACN Sonicated to dissolve and make up volume with diluents.

5-Methyl-3-methylene-hexan-2-one(Imp-6): weighed 20 mg of 5-Methyl-3-methylene-hexan-2-one into 100 ml volumetric flask. Add 5 ml ACN Sonicated to dissolve and make up volume with diluents.

1,3,4,6,7,11b-Hexahydro-9,10-dimethoxy-3-(2-methylpropyl)-2H-benzo[a]quinolizin-2-one(Imp-7): weighed 20 mg of 1,3,4,6,7,11b-Hexahydro-9, 10-dimethoxy-3-(2-methylpropyl)-2H-benzo[a]quinolizin-2-one into 100 ml volumetric flask. Add 5 ml ACN Sonicated to dissolve and make up volume with diluents.

**Spiked impurity mixture:** (Specification limit of impurity not more than 0.2%)

Weighed 200 mg Tetrabenazine standard into 100 ml volumetric flask. Add 60 ml Diluent, sonicated to dissolve; Spiked 2.00 ml of each impurities into it. And make up volume with Diluent mix well. Filter solution with 0.45 µ PVDF filter.

**As such Sample Preparation:**

Weigh accurately 20 tablets and calculate the average weight. Crush the tablet in to fine powder. Transfer an accurately weighed quantity of tablet powder equivalent to about 100 mg of (approx. 520 mg of powder) in to a 50 ml volumetric flask. Add 35 ml of diluent and sonicate for 45 min. With occasional shaking. Cool and make volume up to mark with diluent and mix. Filter the solution through 0.45 µ Millipore PVDF syringe filter. Collect the filtrate by discarding first 3 ml of the filter.

**As such Placebo Preparation:**

Weigh & transfer 429.6 mg of Tetrabenazine placebo into 50 ml volumetric flask. Add 35 ml of Diluent, sonicate for 45 minutes, make up volume with Diluent Mix well. Filter with 0.45µ PVDF filter

**Chromatographic Conditions**

The Inertsil ODS 3V (250 mm x 4.6 mm, 5 µm) was used as the stationary phase. Elution was Gradient by Mobile phase A: Buffer: Acetonitrile (90:10%) where Buffer: di Ammonium hydrogen phosphate pH adjusted by OPA pH of Buffer: 7.1 ± 0.05 and Mobile phase B: Acetonitrile (100%). It

was filtered through 0.45µ (micron) membrane filter and degassed. The mobile phase was pumped at 1.0 ml/min. The eluents were monitored at 224 nm. The injection volumes of sample and standard were 20 µl Total run time was 70 min. Column temperature was 25ºC. Chromatograms are shown in figure:2.

**Table 1Gradient Program**

| Time (min) | Mobile phase A | Mobile phase B |
|---|---|---|
| 0 | 90 | 10 |
| 10 | 75 | 25 |
| 50 | 25 | 75 |
| 55 | 25 | 75 |
| 60 | 90 | 10 |
| 70 | 90 | 10 |

**Forced Degradation Study**

ICH prescribed stress conditions such as acidic, basic, oxidative, thermal and photolytic stresses were carried out.

**Acid degradation**

Weighed Tetrabenazine Tablet crushed powder equivalent to 100 mg. [about 515.5 mg] into 50 ml volumetric flask.Add 30 ml of diluent; Sonicate 45 min.Add 2 ml 1MHCl, Placed into 60 ºC containing water bath for 1 Hr.Cool. Neutralize with 2 ml of 1MNaOH. Make up volume with 1.0 MHCl.Filter with 0.45µ PVDF filter.Chromatograms are shown in figure: 3.

**Alkali Degradation**

Weighed Tetrabenazine Tablet crushed powder equivalent to 200 mg. [about 525.9 mg] into 50 ml volumetric flask. Add 30 ml of diluents, Sonicate 45 min. Add 2 ml 1MNaOH, Placed into 60 ºC containing water bath for for 2 Hr.Cool. Neutralize with 2 ml of 1M HCl. Make up volume with 1MNaOHFilter with 0.45µ PVDF filter.Chromatogramsare shown in figure:4.

**Peroxide Degradation**

Weighed Tetrabenazine Tablet crushed powder equivalent to 100 mg. [about 517.9mg] into 50 ml volumetric flask. Add 30 ml of diluents, Sonicate 45 min.Add 2 ml 6% $H_2O_2$, Placed into 60ºC containing water bath for for 2 Hr.Cool and make up volume with diluent.Filter with 0.45µ PVDF filter. Chromatograms are shown in figure:5.

**Validation Of Develop Method**

**Linearity**

The linearity for Tetrabenazine is established over the range of LOQ-150% of target concentration. These solutions were injected into the HPLC system and area response of the same was recorded. A plot of concentration Vs Y-intercept of the plot were evaluated.The observations were shown in Table:4,5. According to Specification limit, impurity should not be present more than 0.2 %, so solution of linearity of impurity prepared as following in which 0.2 % of sample concentration considered as 100 % that is 4 ppm (2000 ppm is sample concentration). And from that consideration LOD, 50%, 80%, 100%, 120%, and 150% prepared.

**Preparation of Linearity solutions for all impurities**

Specification limit (**Not more than 0.2%)**

**Stock solution preparation (40.0 ppm)**

20 mg of N-[2-(3,4-dimethoxy phenyl) ethyl] formamide and 3,4-dihydro-6,7-dimethoxy isoquinoline and Tetrabenazine into 100 ml volumetric flask individually. Then add 5 ml of Acetonitrile in each flask & sonicate to dissolve impurities. Then make up volume with diluent (200 ppm). Now transfer 20 ml from stock solution into 100 ml volumetric flask and make up the volume up to mark with diluent and mix well (40.0 ppm).

**Acceptance Criteria**

The correlation coefficient should be more than 0.998.
Curve was shown in Figure: 6&7.

**Method Precision**

According to specification limit of all impurities which is not more than 0.2%, amount of individual impurity will be 4 ppm which is 0.2 % of 2000ppm (sample concentration). For Repeatability sample containing all impurities at 100% level injected for six times and for Intermediate precision Sample containing all impurities at 50%, 100%, and 150% level injected for Intraday precision and Interday precision it is injected in 3 sets.

**Acceptance criteria**

% RSD of peak area should not be more than 5.0%. Data shown in the Table: 6,7,8,9,10,11.

**Accuracy**

According to specification limit of all impurities which is not more than 0.2%, amount of individual impurity will be 4 ppm which is 0.2 % of 2000 ppm (sample concentration). Accuracy is performed in sample at 4 levels which are LOQ, 50%, 100%, and 150% in three sets.

**Acceptance Criteria**

The %Recovery at each level should be 70% to 130% of added concentration.

**Specificity**

According to specificity there should not be any interference of placebo or diluents into the peak of impurity and in to the peak of drug as well, and all impurities should be separated from each other and from drug also. So peak of Impurities as well drug compared with the chromatograph of Placebo. Resolution between impurities and drug observed. Individual impurities also injected at 100% level for confirmation of Retention time of individual impurity. Specificity performed at 100% level of impurities that is 4 ppm.

**Acceptance Criteria**

There should be no interference at the RT of analyte peak.

## RESULTS AND DISCUSSION

The Inertsil ODS 3V (250 mm x 4.6 mm, 5 µm) was used as the stationary phase. Elution was Gradient by Mobile phase A: Buffer: Acetonitrile (90:10%) where Buffer: di Ammonium hydrogen phosphate pH adjusted by OPA pH of Buffer: 7.1 ± 0.05 and Mobile phase B: Acetonitrile (100%). It was filtered through 0.45µ (micron) membrane filter and degassed. The mobile phase was pumped at 1.0 ml/min. The eluents were monitored at 224 nm. The injection volumes of sample and standard were 20 µl Total run time was 70 min. Column temperature was 25ºC.

**Table 2: Linearity Preparation**

| Linearity level | Volume of linearity std stock solution(40ppm) to be taken(ml) | Dilute to volume with diluents | Final concentration(µg/ml) |
|---|---|---|---|
| LOQ | 0.3 | 10 | 1.2 |
| 50% | 0.5 | 10 | 2.0 |
| 80% | 0.8 | 10 | 3.2 |
| 100% | 1.0 | 10 | 4.0 |
| 120% | 1.2 | 10 | 4.8 |
| 150% | 1.5 | 10 | 6.0 |

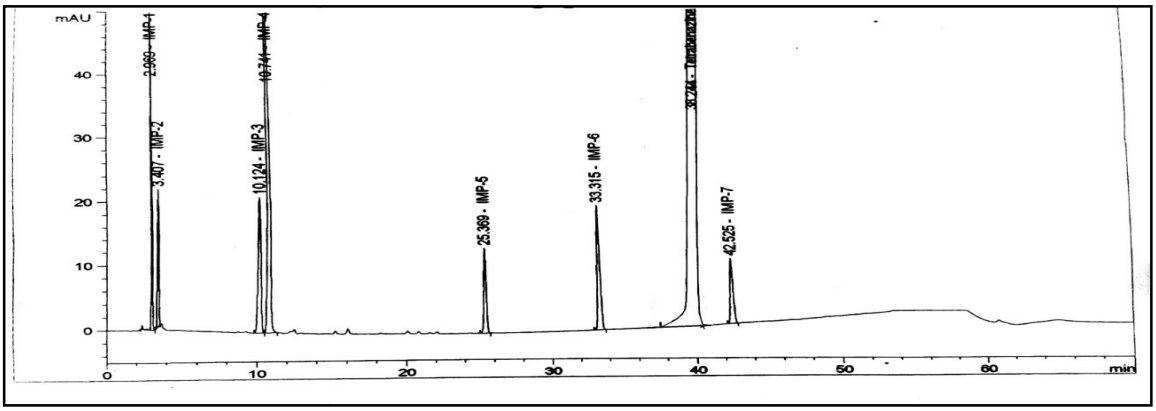


**Figure 2: Chromatogram of Impurity Mixture test sample**

**Table 3: System Suitability Parameters in Optimized condition**

| RT | RRT | Peak name | Plates | Tailing | Resolution |
|---|---|---|---|---|---|
| 2.913 | 0.076 | Imp-1 | 5422 | 0.92 | - |
| 3.354 | 0.087 | Imp-2 | 5683 | 0.97 | 3.125 |
| 10.129 | 0.264 | Imp-3 | 6771 | 1.02 | 29.27 |
| 10.744 | 0.280 | Imp-4 | 7530 | 1.02 | 2.551 |
| 25.384 | 0.663 | Imp-5 | 4723 | 0.99 | 77.00 |
| 33.354 | 0.872 | Imp-6 | 5771 | 1.06 | 36.22 |
| 38.244 | - | Tetrabenazine | 17924 | 0.95 | 20.13 |
| 42.569 | 1.113 | Imp-7 | 16568 | 1.00 | 16.97 |

**Forced Degradation**

**Acid Degradation**

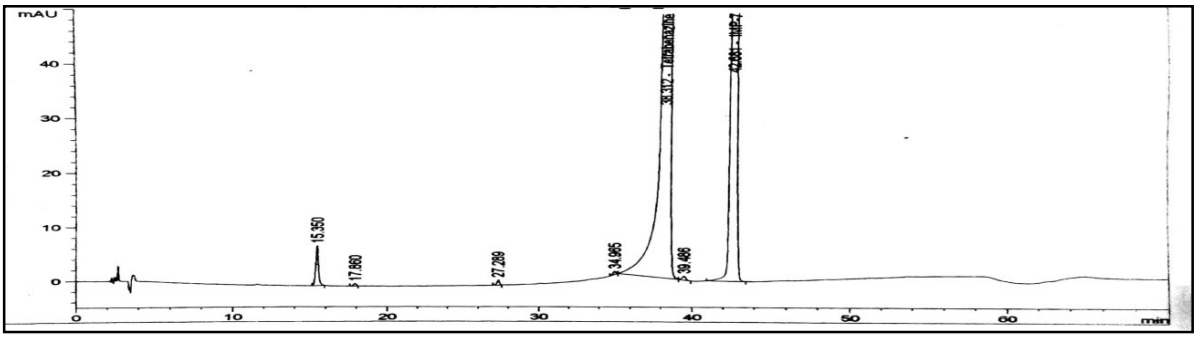


**Figure 3: 1M HCL_2ml_60ºC_1Hr**

**Base Degradation**

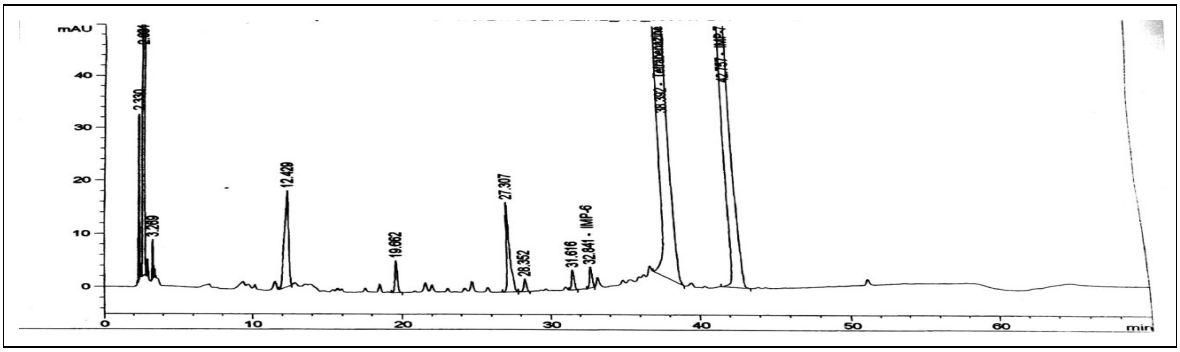


**Figure 4: 1M NaOH_2ml_60ºC_2 Hr**

**Oxidation Degradation**

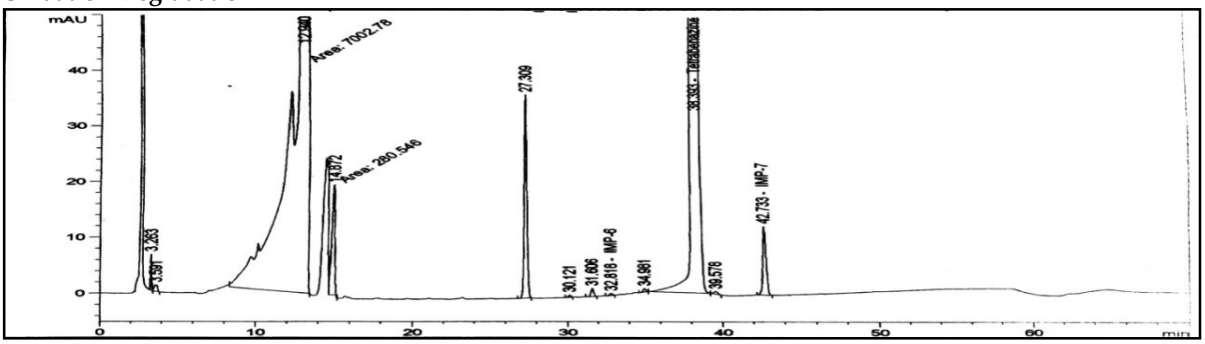


**Figure 5: Peroxide Degradation**

**Method validation**

The described method has been validated which include parameters like System Suitability, Linearity, Accuracy, Precision, Robustness, LOD (limit of detection) and LOQ (limit of quantification).

**Linearity**

The linearity of this method was evaluated by linear regression analysis and calculated by the least square method and studied by preparing standard solutions of Tetrabenazineat different concentration levels. The calibration curve showed in (Fig. 6, 7) good linearity in the range of 1.2-6.0µg/ml with a correlation coefficient ($r^2$) of 0.999. The results are given in table 4, 5.

**Table 4: Linearity result of Imp-3 (N-[2-(3,4-dimethoxyphenyl)ethyl]formamide)**

| Level | Concentration(µg/ml) | Area |
|---|---|---|
| **LOQ** | 1.18 | 81815 |
| **50%** | 1.99 | 138410 |
| **80%** | 3.19 | 214830 |
| **100%** | 3.98 | 269187 |
| **120%** | 4.78 | 323621 |
| **150%** | 5.98 | 393531 |

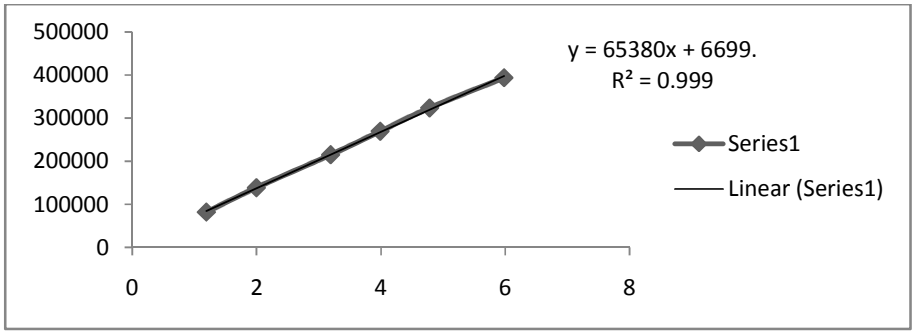


**Figure 6: Calibration curve of Imp-3 (N-[2-(3,4-dimethoxyphenyl)ethyl]formamide)**

**Table 5: Linearity result of Imp-4 (3,4-dihydro-6,7-dimethoxyisoquinoline)**

| Level | Concentration (µg/ml) | Area |
|---|---|---|
| **LOQ** | 1.18 | 144041 |
| **50%** | 1.99 | 228220 |
| **80%** | 3.19 | 367061 |
| **100%** | 3.98 | 461238 |
| **120%** | 4.78 | 575177 |
| **150%** | 5.98 | 700647 |

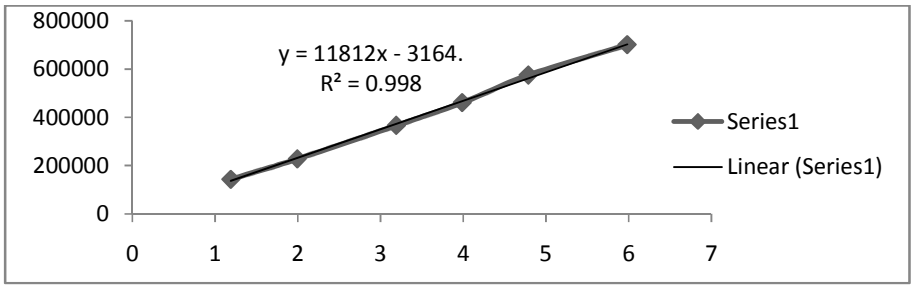


**Figure 7: Calibration curve of Imp-4 (3,4-dihydro-6,7-dimethoxyisoquinoline)**

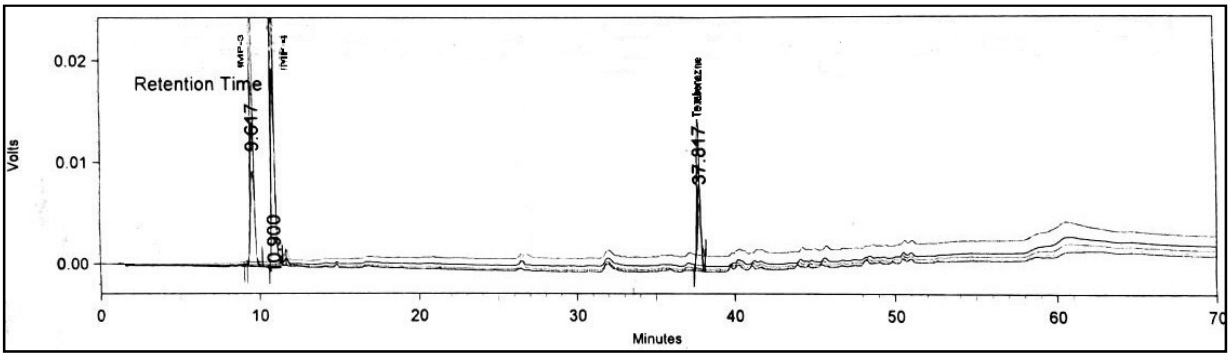


**Figure 8: Linearity overlay Chromatograph for known impurity**

**Precision / Repetability**

**Table 6: Repeatability result of IMP-3**

| Sr. No | Area | Mean | SD | RSD |
|---|---|---|---|---|
| 1 | 265297 | 261859 | 4912.11 | 1.87 |
| 2 | 259195 | | | |
| 3 | 254795 | | | |
| 4 | 264595 | | | |
| 5 | 259277 | | | |
| 6 | 267997 | | | |

**Table 7: Repeatability result of IMP-4**

| Sr. No | Area | Mean | SD | RSD |
|---|---|---|---|---|
| 1 | 461147 | 458297 | 4932.54 | 1.07 |
| 2 | 456957 | | | |
| 3 | 454591 | | | |
| 4 | 460229 | | | |
| 5 | 451567 | | | |
| 6 | 465295 | | | |

**Intermediate precision:**

**Table 8: Intraday precision result of Imp-3**

| For 50 % Level | | | | | | |
|---|---|---|---|---|---|---|
| **Set** | **Level** | **Morning** | **Evening** | **Mean** | **SD** | **RSD** |
| 1 | 50% | 136915 | 137214 | 137064.5 | 211.4249 | 0.15 |
| 2 | 50% | 136714 | 137297 | 137005.5 | 412.2433 | 0.30 |
| 3 | 50% | 137261 | 136115 | 136688 | 810.3444 | 0.59 |
| **For 100 % Level** | | | | | | |
| **Set** | **Level** | **Morning** | **Evening** | **Mean** | **SD** | **RSD** |
| 1 | 100% | 269510 | 268720 | 269115 | 558.6147 | 0.22 |
| 2 | 100% | 269127 | 270179 | 269653 | 743.8763 | 0.27 |
| 3 | 100% | 268790 | 267920 | 268355 | 615.1829 | 0.23 |
| **For 150 % Level** | | | | | | |
| **Set** | **Level** | **Morning** | **Evening** | **Mean** | **SD** | **RSD** |
| 1 | 150% | 391521 | 393740 | 392630.5 | 1569.07 | 0.40 |
| 2 | 150% | 395211 | 392450 | 393830.5 | 1952.32 | 0.49 |
| 3 | 150% | 394691 | 392920 | 393805.5 | 1252.28 | 0.32 |

**Table 9: Intraday precision result of Imp-4**

| For 50 % Level | | | | | | |
|---|---|---|---|---|---|---|
| **Set** | **Level** | **Morning** | **Evening** | **Mean** | **SD** | **RSD** |
| 1 | 50% | 226970 | 227949 | 227459.5 | 692.2575 | 0.30 |
| 2 | 50% | 230240 | 229234 | 229737 | 711.3494 | 0.32 |
| 3 | 50% | 228642 | 227867 | 228254.5 | 548.0078 | 0.24 |
| **For 100 % Level** | | | | | | |
| **Set** | **Level** | **Morning** | **Evening** | **Mean** | **SD** | **RSD** |
| 1 | 100% | 468749 | 467475 | 468112 | 900.85 | 0.19 |
| 2 | 100% | 465651 | 467479 | 466565 | 1292.591 | 0.27 |
| 3 | 100% | 462475 | 464561 | 463518 | 1475.025 | 0.32 |
| **For 150 % Level** | | | | | | |
| **Set** | **Level** | **Morning** | **Evening** | **Mean** | **SD** | **RSD** |
| 1 | 150% | 701969 | 705496 | 703732.5 | 2493.966 | 0.35 |
| 2 | 150% | 706976 | 710796 | 708886 | 2701.148 | 0.38 |
| 3 | 150% | 710945 | 706973 | 708959 | 2808.628 | 0.40 |

**Table 10: Interday precision result of Imp-3**

| For 50 % Level | | | | | | |
|---|---|---|---|---|---|---|
| **Set** | **Level** | **Day-1** | **Day-2** | **Mean** | **SD** | **RSD** |
| 1 | 50% | 138541 | 139125 | 138838 | 412.95 | 0.29 |
| 2 | 50% | 138621 | 138254 | 138437.5 | 259.50 | 0.19 |
| 3 | 50% | 138047 | 138476 | 138261.5 | 303.34 | 0.22 |
| **For 100 % Level** | | | | | | |
| **Set** | **Level** | **Day-1** | **Day-2** | **Mean** | **SD** | **RSD** |
| 1 | 100% | 269215 | 267540 | 268377.5 | 1184.404 | 0.44 |
| 2 | 100% | 266148 | 268299 | 267223.5 | 1520.987 | 0.57 |
| 3 | 100% | 267995 | 270577 | 269286 | 1825.75 | 0.67 |
| **For 150 % Level** | | | | | | |
| **Set** | **Level** | **Day-1** | **Day-2** | **Mean** | **SD** | **RSD** |
| 1 | 150% | 392733 | 389543 | 391138 | 2255.67 | 0.57 |
| 2 | 150% | 394824 | 391945 | 393384.5 | 2035.76 | 0.52 |
| 3 | 150% | 389984 | 393951 | 391967.5 | 2805.09 | 0.72 |

**Table 11: Interday precision result of Imp-4**

| For 50 % Level | | | | | | |
|---|---|---|---|---|---|---|
| **Set** | **Level** | **Day-1** | **Day-2** | **Mean** | **SD** | **RSD** |
| 1 | 50% | 228294 | 228975 | 228634.5 | 481.54 | 0.21 |
| 2 | 50% | 228595 | 229165 | 228880 | 403.05 | 0.17 |
| 3 | 50% | 229079 | 228367 | 228723 | 503.46 | 0.22 |
| **For 100 % Level** | | | | | | |
| **Set** | **Level** | **Day-1** | **Day-2** | **Mean** | **SD** | **RSD** |
| 1 | 100% | 462749 | 465975 | 464362 | 2281.12 | 0.49 |
| 2 | 100% | 464761 | 467149 | 465955 | 1688.57 | 0.36 |
| 3 | 100% | 463675 | 466479 | 465077 | 1982.72 | 0.42 |
| **For 150 % Level** | | | | | | |
| **Set** | **Level** | **Day-1** | **Day-2** | **Mean** | **SD** | **RSD** |
| 1 | 150% | 701776 | 704946 | 703361 | 2241.52 | 0.32 |
| 2 | 150% | 705226 | 709545 | 707385.5 | 3053.99 | 0.43 |
| 3 | 150% | 710462 | 705787 | 708124.5 | 3305.72 | 0.47 |

**Accuracy**

Accuracy of the method was confirmed by recovery study of Tetrabenazine at 4 levels (LOQ,50%, 100%, 150%) by standard addition method.The results are given in table 12, 13.

**Table 12: Recovery result of Imp-3**

| **Set** | **Level** | **Amount Added (µg/ml)** | **Area** | **Amount Found (µg/ml)** | **% Recovery** | **%Mean Re-covery** | **SD** | **RSD** |
|---|---|---|---|---|---|---|---|---|
| 1 | LOQ | 1.2 | 81555 | 1.19 | 99.93 | 99.93 | 0.61 | 0.61 |
| 2 | LOQ | 1.2 | 80575 | 1.18 | 98.73 | | | |
| 3 | LOQ | 1.2 | 81245 | 1.19 | 99.55 | | | |
| 1 | 50% | 2.0 | 129720 | 1.90 | 95.37 | 95.08 | 0.87 | 0.91 |
| 2 | 50% | 2.0 | 130265 | 1.91 | 95.77 | | | |
| 3 | 50% | 2.0 | 127990 | 1.88 | 94.10 | | | |
| 1 | 100% | 4.0 | 252759 | 3.71 | 92.92 | 91.96 | 0.89 | 0.97 |
| 2 | 100% | 4.0 | 249767 | 3.67 | 91.82 | | | |
| 3 | 100% | 4.0 | 247927 | 3.64 | 91.14 | | | |
| 1 | 150% | 6.0 | 369971 | 5.44 | 90.67 | 92.14 | 1.34 | 1.45 |
| 2 | 150% | 6.0 | 380692 | 5.59 | 93.30 | | | |
| 3 | 150% | 6.0 | 377241 | 5.54 | 92.45 | | | |

**Table 13: Recovery result of Imp-4**

| **Set** | **Level** | **Amount Added (µg/ml)** | **Area** | **Amount Found (µg/ml)** | **% Recovery** | **%Mean Re-covery** | **SD** | **RSD** |
|---|---|---|---|---|---|---|---|---|
| 1 | LOQ | 1.2 | 137579 | 1.18 | 98.36 | 97.57 | 0.96 | 0.98 |
| 2 | LOQ | 1.2 | 134971 | 1.15 | 96.49 | | | |
| 3 | LOQ | 1.2 | 136879 | 1.17 | 97.86 | | | |
| 1 | 50% | 2.0 | 215520 | 1.84 | 92.45 | 91.89 | 1.72 | 1.87 |
| 2 | 50% | 2.0 | 209723 | 1.79 | 89.96 | | | |
| 3 | 50% | 2.0 | 217452 | 1.86 | 93.27 | | | |
| 1 | 100% | 4.0 | 452739 | 3.88 | 97.10 | 96.60 | 0.56 | 0.58 |
| 2 | 100% | 4.0 | 450925 | 3.86 | 96.71 | | | |
| 3 | 100% | 4.0 | 447524 | 3.83 | 95.98 | | | |
| 1 | 150% | 6.0 | 690649 | 5.92 | 98.75 | 98.07 | 0.84 | 0.86 |
| 2 | 150% | 6.0 | 679256 | 5.82 | 97.12 | | | |
| 3 | 150% | 6.0 | 687704 | 5.90 | 98.33 | | | |

**Table 14: System Suitability parameters in which resolution of all impurities shown**

| RT | RRT | Peak name | Plates | Tailing | Resolution |
|---|---|---|---|---|---|
| 2.913 | 0.076 | Imp-1 | 5422 | 0.92 | - |
| 3.354 | 0.087 | Imp-2 | 5683 | 0.97 | 3.125 |
| 10.129 | 0.264 | Imp-3 | 6771 | 1.02 | 29.27 |
| 10.744 | 0.280 | Imp-4 | 7530 | 1.02 | 2.551 |
| 25.384 | 0.663 | Imp-5 | 4723 | 0.99 | 77.00 |
| 33.354 | 0.872 | Imp-6 | 5771 | 1.06 | 36.22 |
| 38.244 | - | Tetrabenazine | 17924 | 0.95 | 20.13 |
| 42.569 | 1.113 | Imp-7 | 16568 | 1.00 | 16.97 |

**Impurity Mixture**

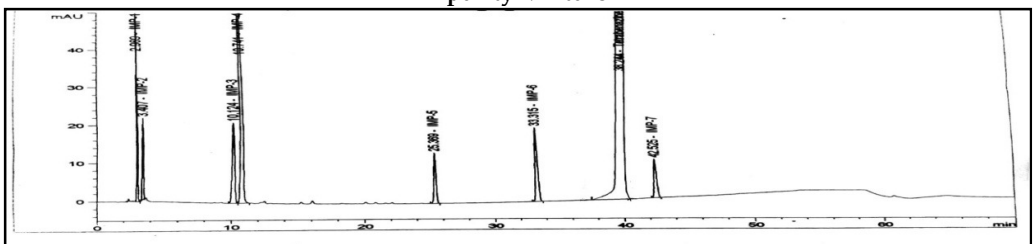

**Figure 9: Impurity Mixture**

Tetrabenazine undergoes significant degradation in acid, base, peroxide, thermal and UV. Comparatively, more degradation was found with PeroxideDegradation. Forced Degradation Summary is given in Table 15.

**Table 15: Forced Degradation Summary**

| Sr. No. | Stress Condition | Duration | Area | % Degradation | % Mass Balance |
|---|---|---|---|---|---|
| 1 | Acid Hydrolysis (1M HCl_2ml) | 60°C for 1 HR | 497746 | 2.15 | 99.63 |
| 2 | Base Hydrolysis (1M NaOH_2ml) | 60°C for 2 HR | 489759 | 3.70 | 100.06 |
| 3 | Peroxide degradation (6%$H_2O_2$_1ml) | 60°C for 2 HR | 469712 | 7.65 | 100.79 |

Hence, a method of the determination of Tetrabenazine with its known and unknown degradation impurities in its tablet dosage form shows that the degradation product doesn't interfere with the analytical determination. Hence the proposed analytical method is also useful for the determination of Tetrabenazine with its known and unknown degradation impurities in a sample of the pharmaceutical dosage form.

## CONCLUSION

All the parameter and results were found within the acceptance limit as given in the Validation protocol. So we can conclude that developed RP-HPLC Method was Selective, specific, sensitive, linear, accurate, precise, and robustness. Therefore method is found to be specific for Tetrabenazine related substances with good resolution. It can be applied for the forced degradation study. So the proposed method can be used in pharmaceutical analysis for Forced degradation study and routine quality control sample of Terabenazine Tablet.

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## REFERENCES

1. "Tetrabenazine Drug Profile" September 2017, https://www.drugbank.ca/drugs/DB04844

2. "Tetrabenazine Drug Profile" September 2017, https://pubchem.ncbi.nlm.nih.gov/compound/tetrabenazine#section=Top
3. "Tetrabenazine Drug Profile" September 2017, http://www.chemspider.com/Chemical-Structure.5796.htm
4. SethiPD, HPLC Quantitative Analysis of Pharmaceutical Formulations, 3rdEdn, CBS Publication and Distributors New Delhi, 2001,pp 1-20, 99-105.
5. U.S. EPA, Guidance for Method development and Method Validation for the Resource Conservation and Recovery Act (RCRA) Program.1995, www.epa.gov/sw846/pdfs/methdev.pdf
6. Hamilton RJ, Sevell PA, Introduction to HPLC, 2ndEdn, Chapman and Hall London Publication 1982, pp 189.
7. Beckett A.H, and Stenlake J. B, Practical Pharmaceutical Chemistry, 1stEdn, CBS Publishers and Distributors New Delhi, 2004, pp 275-325.
8. Dr. S. Ravi Shankar, Textbook of Pharmaceutical Analysis, 3rdEdn, RX Publication, 2014, pp13.1, 13.3, 18.2 and 18.5.
9. Vogel's, Textbook of Quantitative Chemical Analysis, 5th Edn, Pearson Education Limited Publication, 1996, pp 645-651, 752-755.
10. Sharma S, Sher P, Badve S, Atmaram P.P, Recent Advances in Spectroscopic techniques, 6thEdn, AAPS Pharm Science Technology Publication,2005, pp 618-625.
11. ICH Harmonized tri partitle guideline, Validation of Analytical Procedures: Text and Methodology, Q2 (R1), 2005, pp 8-17.
12. Ahuja s, and Dong MW, Handbook of Pharmaceutical Analysis by HPLC, 1stEdn, Elsevier Academic Press, United Kingdom, 2005, pp 62-68.
13. Singh SS and Bakshi M, Development of validated stability indicating assay methods: critical review. Journal of Pharmaceutical and biomedical analysis, April 2002, pp11–40.
14. Schirmer RE, Modern Methods of Pharmaceutical Analysis, CRC Press Inc, 1982, pp61-138.
15. Cartenson JT, and Swarbirck J, Solution kinetics-Drug stability principles and practice, 2ndEdn, Marcel Dekker series, 1995, pp 17-90.
16. Stability Testing of New Drugs and Product:ICH Q1 A (R2). 2003, pp 1-20.
17. Charde MS, Kumar J, Welankiwar AS and Chakole RD, Review: development of forced degradation studies of drugs. Int J of Advances in P'ceutics,December 2013, pp34-38.
18. Validation of Analytical Procedure: Text and Methodology ICH, Q2 (R1).2005, pp 1-13.
19. Patel PMM N, Mehta I, Patel CJ, Stability Indicating Method For the Simultaneous Estimation of Cefuroxime Axetil and Linezolid in Pharmaceutical Dosage Forms. International Journal of Pharmaceutical & Drug Analysis, 2017, 5(12), pp456-466.
20. Hotha KK, Reddi SK, Raju VK and Ravindranath LK, Forced degradation studies: practical approach- overview of regulatory guidance and literature for the drug substances and drug products. Int Research J of Pharm, July2013, pp78-83.