

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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Date Received: 24th March 2018; Date accepted:
11th April 2018; Date Published: 19th April 2018

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 25°C; 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. Tetrabenazine is 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.

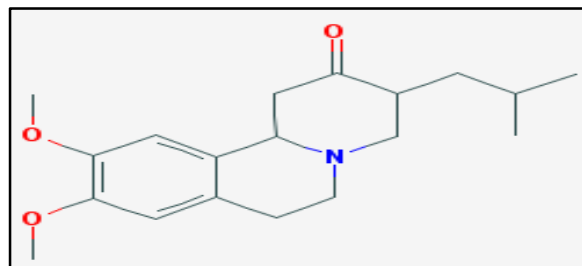


Figure1 Tetrabenazine structure

MATERIALS AND METHODS^[4-20]

Material and Reagents

Acetonitrile, di Potassium Hydrogen Phosphate, di Ammonium hydrogen phosphate, Ammonium acetate, Ortho Phosphoric 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 10 μ l 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 up 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 0.45 μ PVDF filter

Chromatographic Conditions

The Inertsil ODS 3V (250 mm x 4.6 mm, 5 μ m) 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 \pm 0.05 and Mobile phase B: Acetonitrile (100%). It

was filtered through 0.45 μ (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 μ l Total run time was 70 min. Column temperature was 25°C. Chromatograms are shown in figure:2.

Table 1 Gradient 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 1M HCl, Placed into 60 °C containing water bath for 1 Hr. Cool. Neutralize with 2 ml of 1M NaOH. Make up volume with 1.0 M HCl. Filter with 0.45 μ 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 1M NaOH, Placed into 60 °C containing water bath for for 2 Hr. Cool. Neutralize with 2 ml of 1M HCl. Make up volume with 1M NaOH. Filter with 0.45 μ PVDF filter. Chromatograms are 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% H₂O₂, Placed into 60°C containing water bath for for 2 Hr. Cool and make up volume with diluent. Filter with 0.45 μ PVDF fil-

ter. 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 25°C.

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

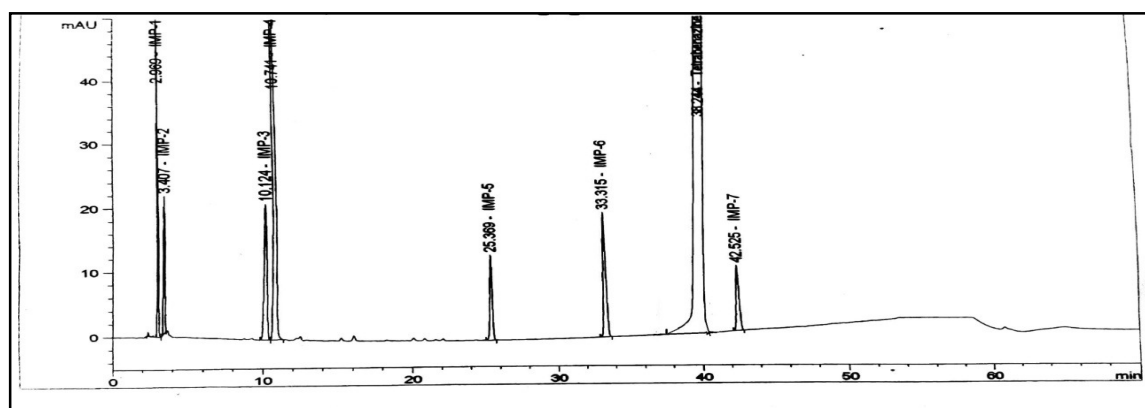


Figure 2: Chromatogram of Impurity Mixture test sample

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

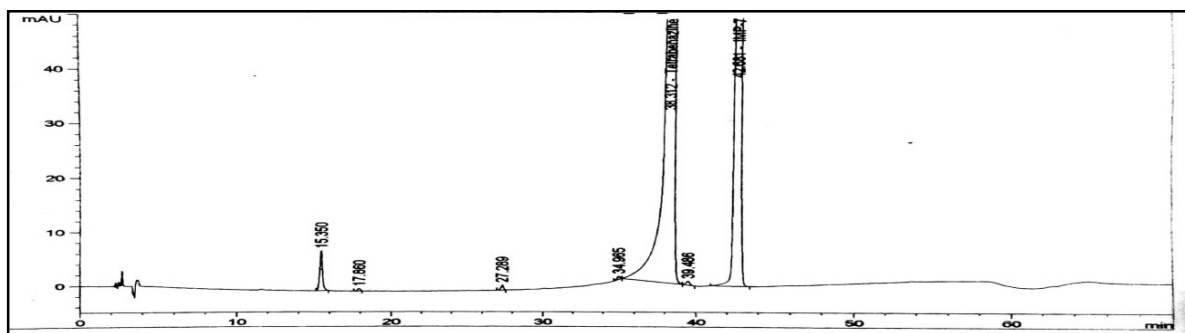


Figure 3: 1M HCL_2ml_60°C_1Hr

Base Degradation

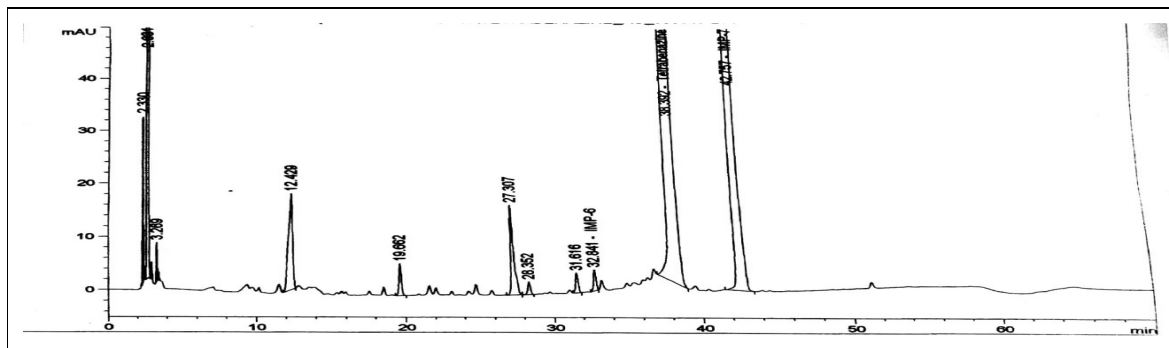


Figure 4: 1M NaOH_2ml_60°C_2 Hr

Oxidation Degradation

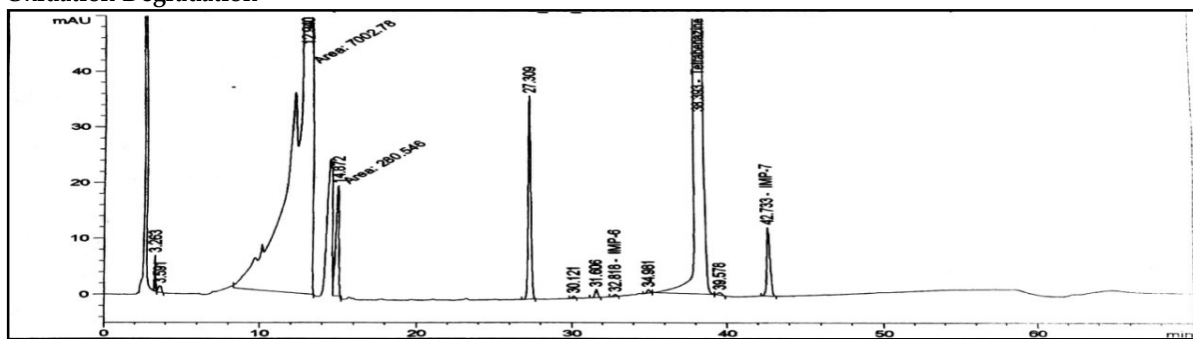


Figure 5: Peroxide Degradation

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 Tetrabenazine at different concentration levels. The calibration curve showed in (Fig. 6, 7) good linearity in the range of 1.2-6.0 µg/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

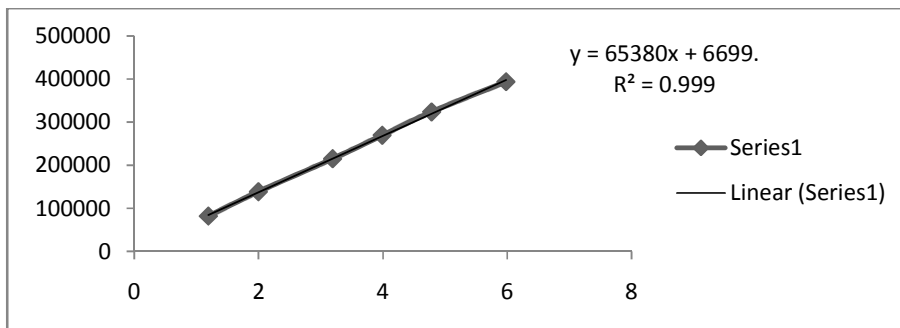


Figure 6: Calibration curve of Imp-3 (N-[2-(3,4-dimethoxyphenyl)ethyl]formamide)

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

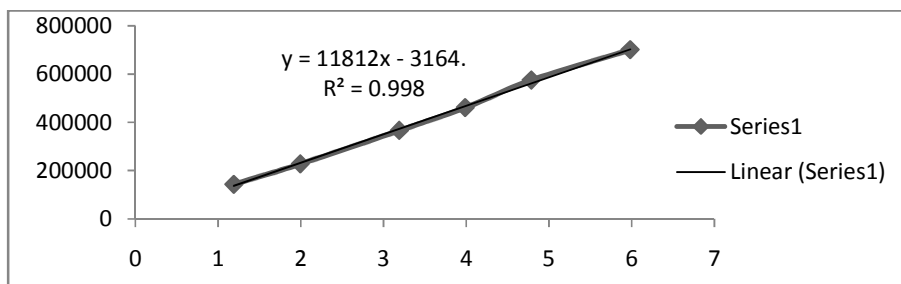


Figure 7: Calibration curve of Imp-4 (3,4-dihydro-6,7-dimethoxyisoquinoline)

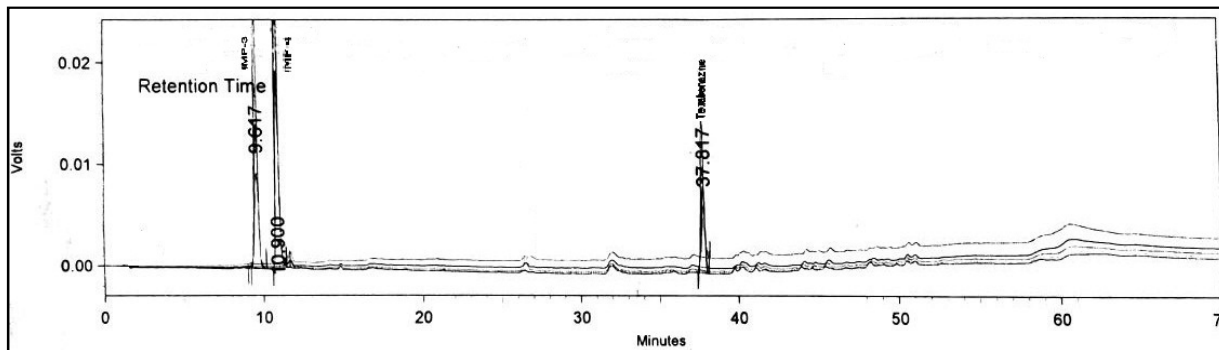


Figure 8: Linearity overlay Chromatograph for known impurity

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 Recovery	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 Recovery	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
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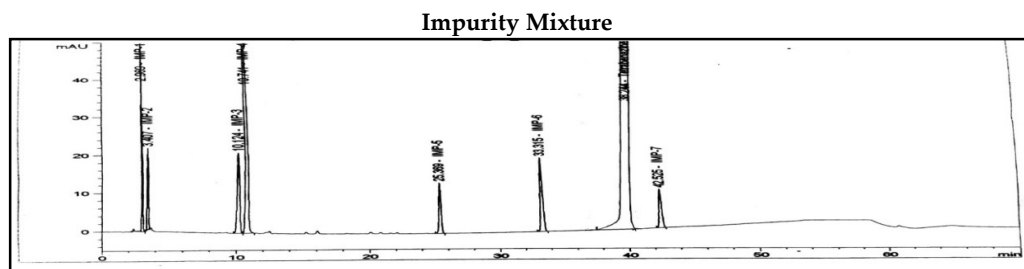


Figure 9: Impurity Mixture

Tetrabenazine undergoes significant degradation in acid, base, peroxide, thermal and UV. Comparatively, more degradation was found with Peroxide Degradation. 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 ₂ O 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 Tetrabe-

nazine 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 Tetrabenazine Tablet.

ACKNOWLEDGEMENT

The authors are thankful to Zydus cedilla, moraiya, Ahmedabad for providing the standard and sample of Tetrabenazine and to provide all the facilities to complete the research work.

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