Development and Validation of Stability Indicating Assay Method for Tetrabenazine in Tetrabenazine Tablets Using HPLC

Shriya.N*[1], Karthik.M[2], Dr. Y. Sridhar Reddy[3], Narendhar.D[4]

1. Department of Pharmaceutical Analysis, Jawaharlal Nehru Technological University, Hyderabad.
2. Analytical Research and Development, Dr. Reddy's Laboratories Ltd, Hyderabad.
3. Department of Clinical Pharmacology and Therapeutics, Nizam's Institute of Medical Sciences, Hyderabad.
4. Department of Pharmaceutics, Kakatiya University, Warangal.

Corresponding Author:

Shriya.N*

Department of Pharmaceutical Analysis,

Jawaharlal Nehru Technological University, Hyderabad.

Abstract

Stability indicating Assay method for Tetrabenazine in Tablet formulation using High-Performance Liquid Chromatography (HPLC) was developed and validated. Method was developed using Waters HPLC and Zorbax SB C18, 250 x 4.6mm, 5µm column. pH 7.5 Phosphate buffer and Methanol in ratio 70:30 % v/v was used as Mobile phase with Isocratic flow and with a Flow rate of 0.8 mL/min. Injection volume was 20 µL and the detection wavelength of 230 nm was chosen. The proposed method was validated according to ICH Q2 guidelines. The developed method demonstrated excellent Linearity ($R^2 = 0.999$), Precision (% RSD was 0.5) and Accuracy (Recovery was between 99.5 % to 100.4 %). The method was also evaluated for its ability to separate Tetrabenazine from its Impurities and Degradation products, confirming to Specificity. The method remained unaffected by small variations in chromatographic conditions hence the method was found to be Robust.

Key Words: Tetrabenazine, Stability indicating Assay, High-Performance Liquid Chromatography, ICH Q2 guidelines.

Introduction

Tetrabenazine, a dopamine-depleting agent used for the treatment of hyperkinetic movement disorders, requires precise quantification in tablet formulations to ensure efficacy and safety. Existing literature methods for Tetrabenazine estimation in oral tablets are limited, prompting the need for a new method that is capable of detecting Tetrabenazine and separating potential impurities that may arise during stability or storage.

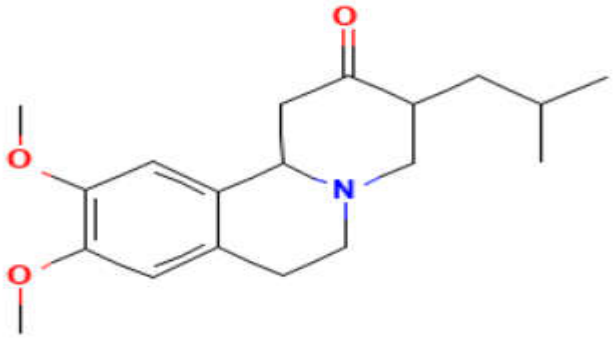


Fig. 1: Structure of Tetrabenazine

Materials and Methods

Chemicals and Reagents:

Tetrabenazine Reference standard, 99.8 % purity was supplied by USP and the coated tablets (Xenazine®) which contained 25 mg of Tetrabenazine were obtained commercially. All the chemical reagents used in this test were Analytical or HPLC grade.

Instrumentation:

Waters HPLC 2965 System with Auto-Injector and PDA detector with the software Empower 3 and Zorbax SB C18, 250 x 4.6mm, 5µm column.

Mobile phase preparation:

The mobile phase was prepared by dissolving 1.74 g of Dipotassium hydrogen phosphate (K2HPO4) in 1000 mL Water, mixed well and adjusted pH to 7.5 ± 0.05 with diluted Orthophosphoric acid solution. Filtered through 0.45 µm membrane filter (Millipore make). Then mixed 700 mL of pH 7.5 buffer and 300 mL of methanol and degassed.

Diluent preparation:

25:75 % Water: Methanol were chosen as diluent to make the method economical and reduce usage of harmful organic solvents.

Selection of Detection Wavelength:

The sensitivity of method that uses UV detector depends upon the proper selection of wavelength that gives maximum absorbance and good response for the given candidate drug. A UV spectrum of Tetrabenazine was recorded by scanning from 190 to 400 nm using methanol as medium. From this method λ max 230 nm was selected for the study.

Chromatographic Conditions:

- Mobile phase: Buffer and Methanol (70:30 % v/v)
- Flow rate: 0.8 mL/min
- Injection volume: 20 µL
- Detection wavelength: 230 nm

Method Development

HPLC method development entails numerous critical processes, including sample pretreatment and band detection, as well as the selection of separation parameters. Method validation is the process of giving recorded evidence that the technique performs as planned. Method validation guarantees that the suggested analytical methodology is accurate, specific, reproducible, and suitable for its intended use. Method validation follows the ICH criteria.

Standard Solution Preparation:

Weighed and transferred 250 mg of Tetrabenazine API into 200 mL volumetric flask. About 100 mL of diluent was added, sonicated for 5 minutes by shaking periodically and diluted up to mark with diluent and mixed well. Centrifuged a portion of above solution at 2000 rpm, in a centrifuge tube with cap for about 10 minutes. Pipetted 5 mL of the clear centrifugate into a 50 mL volumetric flask, diluted to volume with diluent and mixed well.

Test preparation:

Transferred 10 tablets into 200 mL volumetric flask. About 100 mL of diluent was added, sonicated for 30 minutes to dissolve tablets completely by shaking periodically and diluted up to mark with diluent and mixed well. Centrifuged a portion of above solution at 2000 rpm, in a centrifuge tube with cap for about 10 minutes. Pipetted 5 mL of the clear centrifugate into a 50 mL volumetric flask, diluted to volume with diluent and mixed well.

Fig 1: Chromatogram of Standard Preparation

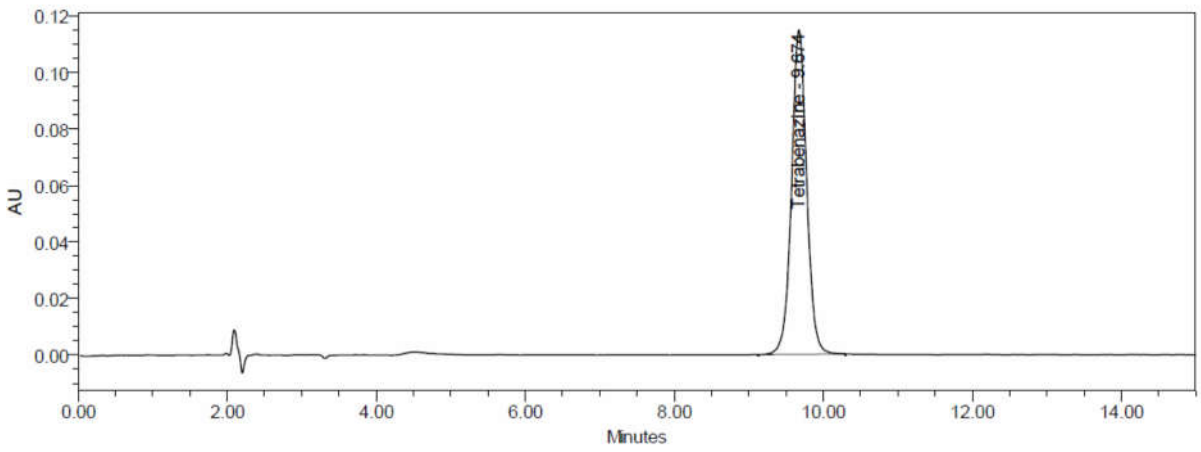
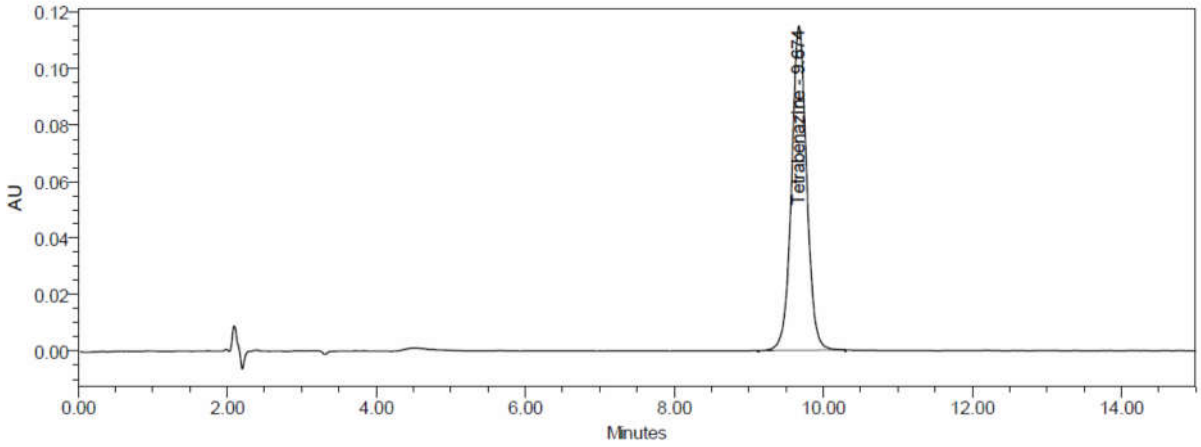


Fig 2: Chromatogram of Sample Preparation



Validation Parameters:

- **System Suitability**: Assessed by injecting standard solutions and evaluating tailing factor, plate count, % RSD of peak areas.
- **Linearity**: Established by analyzing standard solutions at different concentrations and plotting a calibration curve.
- **Precision**: Evaluated through system precision (six injections of standard solution) and method precision (six injections of independent sample preparations).
- **Accuracy**: Determined by recovery studies at various concentration levels.
- **Specificity**: Assessed by analyzing blank, placebo, known impurities and degradation products to ensure no interference with Tetrabenazine peak.
- **Robustness**: Verified by making deliberate changes to chromatographic conditions and evaluating their impact on the assay results.

Results

System Suitability: The system suitability test confirmed that the HPLC system met all the predefined criteria. The tailing factor for Tetrabenazine was 1.03, % RSD for peak area was 0.4, plate count was 11353. (Refer Table 1).

Linearity: The method demonstrated excellent linearity over the concentration range of 10 % to 140 % of Assay test concentration with a Square of correlation coefficient (R^2) 0.999 (Refer Table 3, Figure 3).

Precision: The % RSD for System Precision and Method Precision was 0.5 indicating high precision of the method (Refer Tables 2 and 4).

Accuracy: Recovery studies showed Individual recovery values, Mean recovery values, % RSD within acceptance limits (Refer Table 5).

Specificity: No interference was observed from Blank, Placebo, Impurities, Degradants peaks at the retention time of Tetrabenazine peak demonstrating the specificity of the method (Refer Tables 6,7 and 8).

Robustness: The method remained robust under small deliberate variations in chromatographic conditions and filtered, centrifuged samples (Refer Table 9).

Tables and Figures

Table 1: Results of System Suitability

Parameter	Observed Value	Acceptance Criteria
Tailing factor	1.03	NMT 2.0
% RSD (peak area)	0.4	NMT 2.0
Plate count	11353	NLT 3000

Table 2: Results of System Precision

Injection Number	Peak Area
1	1515529
2	1529091
3	1534941
4	1533657
5	1531823
6	1530785
Mean	**1529304**
% RSD *(NMT 2.0)*	**0.5**

Table 3: Results of Linearity

Concentration (µg/mL)	Peak Area
12.515	153215
25.013	335257
50.06	633843
75.09	976379
100.119	1297113

Concentration (µg/mL)	Peak Area
125.149 (100% level)	1578434
150.179	1926001
175.209	2235960
Square of correlation coefficient (R^2) *(Limit: should not be less than 0.999)*	**0.999**
% Y-intercept at 100% response *(Limit: should be within ±2.0.)*	**0.8**

Figure 3: Linearity Graph of Tetrabenazine

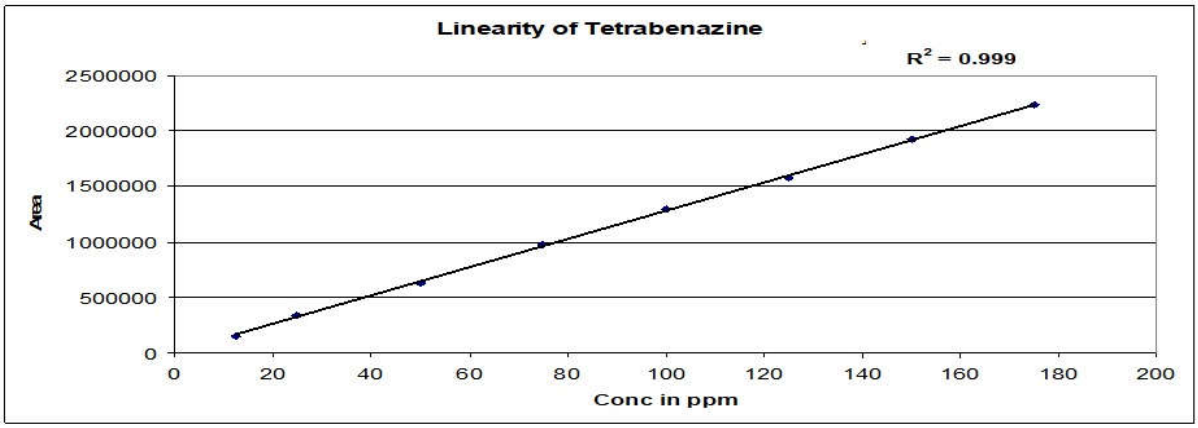


Table 4: Results of Method Precision

Sample Number	% Assay *(Limit: 90.0 to 110.0 %)*
1	101.7
2	100.8
3	101.8
4	101.4
5	100.7
6	101.5
Mean	**101**
% RSD *(NMT 2.0)*	**0.5**

Table 5: Results of Accuracy

% Level	Added Amount (mg)	Found Amount (mg)	% Recovery *(Limit: 98.0 to 102.0)*	Mean % Recovery *(Limit: 98.0 to 102.0)*	% RSD *(NMT 2.0)*
50	12.50	12.48	99.8	99.5	0.3

% Level	Added Amount (mg)	Found Amount (mg)	% Recovery *(Limit: 98.0 to 102.0)*	Mean % Recovery *(Limit: 98.0 to 102.0)*	% RSD *(NMT 2.0)*
	12.42	12.35	99.4		
	12.35	12.25	99.2		
75	18.2	18.26	100.3	99.7	0.7
	18.32	18.29	99.8		
	18.63	18.45	99.0		
100	24.92	24.76	99.4	100.3	0.8
	24.95	25.17	100.9		
	24.60	24.79	100.8		
120	30.25	30.52	100.9	99.9	0.9
	30.55	30.33	99.3		
	30.89	30.75	99.5		
150	37.22	37.35	100.3	100.4	0.2
	37.41	37.52	100.3		
	37.63	37.86	100.6		

Table 6: Results of Blank and Placebo Interference

Sample	Peak Found at RT of Tetrabenazine Peak	Acceptance Criteria
Blank	No	Bank and Placebo should not show any peak at the retention time of Tetrabenazine peak.
Placebo	No	

Table 7: Results of Impurities Interference

Impurity	RT of Impurities (min)	Purity Angle	Purity Threshold	Purity Flag
DHI Impurity	3.388	0.201	0.210	No
Dehydro Impurity	5.538	0.102	0.130	No
3-Keto Impurity	8.621	0.121	0.150	No
N-Butyl Impurity	10.451	0.250	0.286	No
Diastereomer Impurity	12.181	0.150	0.180	No
Tetrabenazine	9.622	0.301	0.310	No

Figure 4: Typical Chromatogram of Test Preparation Spiked With Known Impurities

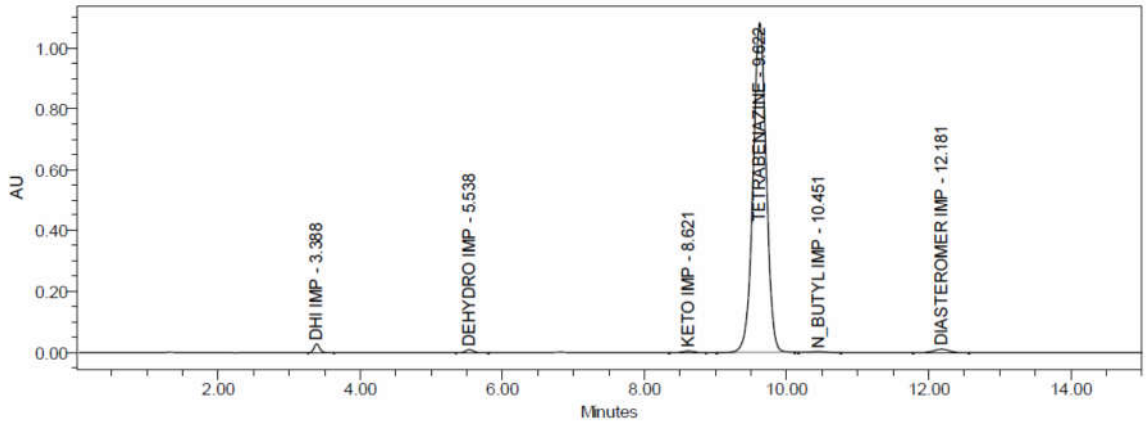


Figure 5: Purity Plot for Test Preparation Spiked With Known Impurities

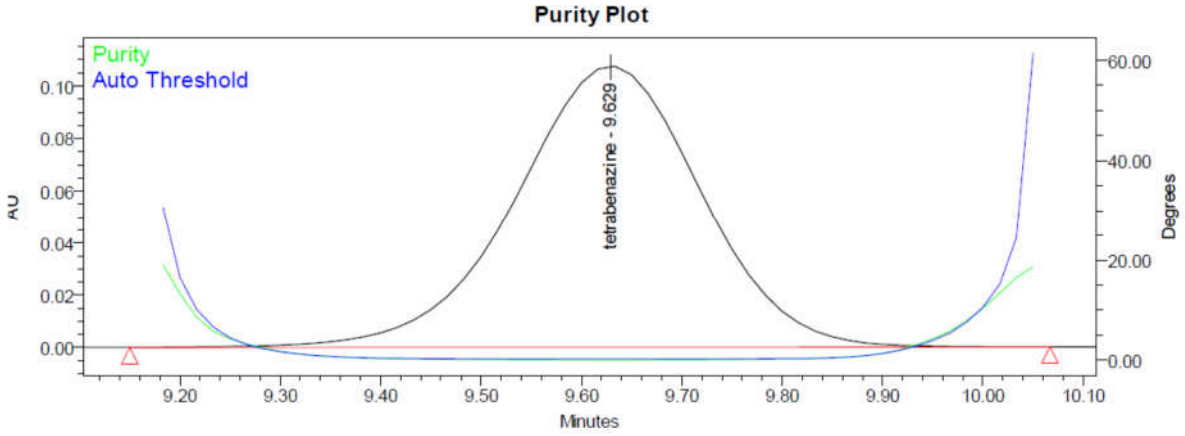


Table 8: Peak Purity Results from Forced Degradation Studies

Stress conditions	% Degradation	Peak purity		Purity Flag	Acceptance Criteria
		Purity angle	Purity threshold		
Degradation in Acid	13.7	0.212	0.260	No	The Purity angle should be less than Purity Threshold and no purity flag for
Degradation in Water	4.0	0.205	0.252	No	
Degradation in Base	21.9	0.233	0.255	No	

Oxidative degradation	7.8	0.221	0.261	No	Tetrabenazine peak
Thermal	3.2	0.230	0.257	No	
Humidity	3.0	0.241	0.265	No	
Photolytic degradation	4.6	0.235	0.257	No	

Table 9: Results of Filter Interference

% Assay			**Difference between Centrifuged and filtered Sample *(NMT ± 2.0)***	
Centrifuged	Sample filtered through PVDF filter	Sample filtered through Nylon 66 filter	Sample filtered through PVDF filter	Sample filtered through Nylon 66 filter
102.4	102.9	102.3	0.5	0.1

Discussion

The developed HPLC method for Tetrabenazine quantification in tablet formulation was validated and met all ICH guidelines. The method was precise, accurate, linear, specific and robust making it suitable for routine quality control and stability studies of Tetrabenazine tablets. The ability to separate Tetrabenazine from its impurities and degradation products ensures the reliability of the assay in various conditions.

The results include responses from chromatograms. Responses of chromatograms gave values of peak area, number of theoretical plates, retention time which are necessary for estimation of Tetrabenazine. Every validation parameter as per ICH guidelines was practically explored and the results and conclusions drawn were detailed in this paper.

System suitability and System Precision showed % RSD (% Relative Standard Deviation) less than 2 concerning peak areas for the drug which indicates the method developed and optimized is system suitable and system precise.

Six injections of the six sample preparations showed % RSD less than 2 concerning % assay and peak areas for the drug which indicates the method developed and optimized was precise.

A study to evaluate the interference from known impurities was conducted by analyzing the test preparation spiked with known impurities at 1.0 % level as per the test method. It was found that the known impurities were not interfering with Tetrabenazine peak. The peak purity of Tetrabenazine in the chromatogram of test preparation spiked with known impurities was found to be within the limit.

A linear relationship between peak areas versus concentrations was observed for Tetrabenazine in the range of 10 % to 140 % of test concentration. The correlation coefficient of Tetrabenazine was 0.999 which met the method validation acceptance criteria.

Accuracy studies revealed that desirable recoveries were achieved (98 – 102%) as per acceptance criteria of method validation. % RSD for Tetrabenazine was less than 2.0. Hence, the method developed and optimized was accurate.

Method developed was found to be robust as it was found that the results of peak performance parameters i.e. Resolution factor (Rs) >2.0, Tailing factor < 2.0 and Number of theoretical plates (Efficiency) more than 3000 were within acceptance criteria despite of deliberate variations done concerning flow rate and pH.

Conclusion

The developed Isocratic RP-HPLC method allows for accurate, precise and reliable measurement of Tetrabenazine in tablet dosage form. The method was evaluated in mass of facets, such as best condition, linear relation including coefficient of correlation, robustness, accuracy, specificity and precision.

The % RSD for all the validation parameters was found to be less than 2, which indicates the validity of method and assay results obtained by this method were in fair agreement as per ICH guidelines. The developed method can be used for routine quantitative estimation of Tetrabenazine in Tablet dosage form.

References

1. Derangula VR, Pilli NR et al. Developed and validated a simple, rapid, and sensitive Huynh-Ba, K., *Handbook of stability testing in pharmaceutical development regulations, methodologies, and best practices*. Springer Verlag, 2008

2. Grimm, W., Storage conditions for stability testing in the EC, Japan and USA; the most important market for drug products. *Drug Development and Industrial Pharmacy* 1993, 19, (20), 2795-2830

3. ICH guideline, Q1A (R2)- Stability testing of new drug substances and products, International Conference on Harmonization, Food and Drug Administration, USA, February, 2003

4. ICH. Guidance for Industry, Q1B "Photostability Testing of New Drug Substances and Product", ICH-Q1B. 1996 November

5. Silke Klick, Pim G. Muijselaar, "toward a generic approach for stress testing of drug substances and drug products", *Journal of Pharmaceutical Technology*, 2005

6. Saranjit Singh and M. Bakshi, Pharmaceutical Technology On-line, 2000; 24: 1-14

7. M. Bakshi and Saranjit Singh. *Journal of Pharmaceutical and Biomedical Analysis*, 2002, 28(6), 1011-40

8. D.W. Reynolds et al., "Available Guidance and Best Practices for Conducting Forced Degradation Studies", *Pharm. Technol*, 2002, 26(2),48–56

9. K.M.Alsante, L.Martin, S.W. Baertschi, "A Stress Testing Benchmarking Study", *Pharm. Technol*, 2003, 27(2),60–72

10. L.R. Snyder, J.J. Kirkland, J.L. Gl ajch, Practi cal HPLC Method Development, Wiley, New York, 1997, 295.

11. Quality assurance of pharmaceuticals, a compendium of guidelines and related materials, Volume 2, 2nd updated edition, Good manufacturing practices and inspection, World Health Organization: 129-131.

12. Guidance for Industry, Q2 (R1) Validation of Analytical Procedures: Methodology, U.S. Department of Health and Human Services, Food and Drug Administration, Center for Drug Evaluation and Research (CDER), Center for Biologics Evaluation and Research (CBER), International Conference on Harmonization, Geneva, November 2005.

13. Ana C.P. Osório, Alessandra L.M.C. da Cunha, Spectrofluorimetric determination of tetrabenazine after photochemical derivatization in basic medium OriginalResearchArticle Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy, Volume100, 1January2013, Pages166-170

14. Mehvar R, Jamali F, Watson MW, Skelton D. Direct injection high-performance liquid chromatography of tetrabenazine and its metabolite in plasma of humans and rats. J Pharm Sci. 1986 Oct;75(10):1006-9.