ISSN: 2231-6078

INTERNATIONAL JOURNAL OF DRUG DISCOVERY AND HERBAL RESEARCH (IJDDHR)

1(3): July-September: (2011), 117-120

Research Article

Development and Validation of Assay Method for Estimation of Amantadine Hydrochloride by HPLC

Nanasaheb Kunjir[1*], Avanija Dube[2] and Sumit Sahu[2]

1, Annasaheb Magar Collage, Hadapsar, Pune, MH, India
2, GRY Institute of Pharmacy, Borawan, Khargone, M.P., India

Abstract

A simple, rapid, accurate and precise HPLC method have been developed for estimation of % drug released of Amantadine Hydrochloride from Amantadine Hydrochloride tablet Dissolution method was developed using C18, 50 mm x 4.6 mm, 5 µm as stationary phase. Buffer: ACN (35:65) was used as mobile phase. The method was validated in terms of linearity, precision, accuracy, specificity and robustness. All the validation was done as per ICH guidelines.

Keywords: Dissolution of Amantadine Hydrochloride, HPLC, ICH.

Introduction

Amantadine Hydrochloride is a White to nearly white crystalline powder, freely soluble in water and soluble in alcohol and chloroform. Drug having efficacy in the treatment of influenza A and as a antiparkinson. Literature survey revealed that, several spectrophotometric, titrimetric and chromatographic methods for estimations of Amantadine Hydrochloride in bulk, biological fluids and in their pharmaceutical formulations. But no method was reported for dissolution development by derivatization method and by HPLC. So here aim was to develop the accurate, precise, rapid and economic assay method for estimation of Amantadine Hydrochloride by HPLC.

Material and Methods

Materials

1. Amantadine hydrochloride: - Working standard and its claimed purity was 98.20%.
2. Amantadine hydrochloride Tablet (label claim 100 mg) and placebo.
3. Di- Sodium Tetra borate Decahydrate
4. 9-Fluroenylmethyl Chloroformate

Reagents and Chemicals

1. Acetonitrile: -HPLC grade, Rankem, India.
2. Methanol: - HPLC grade, Rankem, India.
3. Milli-Q water: - It was purified by Millipore Corporation's system.

Instruments, Apparatus and equipment

1. High Performance Liquid chromatography system (HPLC): Waters Liquid Chromatography with PDA detector
2. Chromatographic software:– Empower
3. A double beam UV-visible spectrophotometer having two matched cells with 1cm light path: - Perkin Elmer
4. Analytical Balance: - XS205, Mettler Toledo, Schwerzenland.
5. pH Meter: - S20k, Mettler
6. Dissolution Tester Apparatus:- Elecrolab

***Corresponding Author**
E-mail: nanakunjir@rediffmail.com,
Mob. +91 9630270986

Analytical Method:

Dissolution Parameters:

Medium	: Deaerated Water
Apparatus	: Paddle
Speed	: 50 RPM
Time Interval	: 45 minutes
Temperature	: 37 ± 0.5°C
Withdrawal & replenishment volume	: 10 mL
Media Volume	: 500 mL

Preparation of Disodium tetra borate decahydrate solution:
Weigh accurately 19.1 g of Di- Sodium Tetra borate Decahydrate (Merck) to a 1000 mL volumetric flask. Add 750 mL of water & Sonicate to dissolve. Dilute to volume with water and mix.

Preparation of 9-Fluroenylmethyl Chloroform ate Solution :
Weigh accurately 4.0 g of 9-Fluroenylmethyl Chloroformate (Merck) to a 1000 mL volumetric flask. Add 750 mL of acetonitrile & Sonicate to dissolve. Dilute to volume with acetonitrile and mix.

Sample Preparation :
Perform the test on six tablets. Place one tablet in each of the dissolution vessel containing 500 mL of medium maintained at 37 ± 0.5°C. Run the instrument. At the above specified time interval, withdraw an aliquot. Filter through 0.45 µ nylon membrane syringe filter discarding initial 2 mL of filtrate. Dilute 5mL of this filtrate to 100mL dissolution medium.

Preparation of Sample Solution by derivatization :
Transfer 5 mL of filtered sample solution into a 25 mL of volumetric flask. Add 5 mL of diluent, 5 mL of Di- Sodium Tetra borate Decahydrate Solution and 5 mL of 9-Fluroenylmethyl Chloroformate Solution. Shake well and allow the solution to stand for 20 minutes. Dilute to volume with diluent and mix.

Chromatographic Conditions :

Instrument	: HPLC
Column	: C18, 50 mm x 4.6 mm, 5 µm
Wavelength	: 210 nm
Injection Volume	: 100 µL
Run Time	: 15 minutes
Column oven temperature	: 30°C

Buffer Preparation :
Weigh & dissolve 1.36 g of Potassium dihydrogen orthophosphate in 1000 mL of Water. Filter through 0.45 µ nylon membrane filter.

Mobile Phase Preparation :
Prepare degassed mixture of Buffer: Acetonitrile in the ratio 35:65. Adjust pH to 3.0 ± 0.05 with Orthophosphoric acid.

Diluent Preparation :
Mix water and Acetonitrile in the ratio 30: 70. Adjust pH to 13.0 ± 0.05 with sodiu hydroxide solution.

Preparation of Diluent blank Solution by derivatization:
Transfer 5 mL of dissolution medium into a 25 mL of

volumetric flask. Add 5 mL of diluent, 5 mL of Di-Sodium Tetra borate Decahydrate Solution and 5 mL of 9-Fluroenylmethyl Chloroformate Solution shake well and allow the solution to stand for 20 minutes. Dilute to volume with diluent and mix.

Preparation of Amantadine Hydrochloride Standard stock solution:

Weigh accurately about 50 mg of Amantadine Hydrochloride working standard to a 250 mL volumetric flask. Add 200 mL of dissolution medium & sonicate to dissolve. Dilute to volume with dissolution medium and mix. Further transfer 5 mL of solution to 100 mL volumetric flask and dilute to volume with dissolution medium.

Preparation of Amantadine Hydrochloride Standard Solution by derivatization :

Transfer 5 mL of Amantadine Hydrochloride standard stock solution into a 25 mL of volumetric flask add 5 mL of diluent, 5 mL of Di- Sodium Tetra borate Decahydrate Solution and 5 mL of 9-Fluroenylmethyl Chloroformate Solution shake well and allow the solution to stand for 20 minutes. Dilute to volume with diluent and mix.

Procedure: Injection sequence:-

Sr. No	Description	No of Injection
1	Blank	01
2	Standard solution	05
3	Test solution 1-6	1 of each
4	Bracketing standard	01

Calculation:

% of Amantadine Hydrochloride dissolved =

$$\frac{A}{B} \times \frac{C}{250} \times \frac{5}{100} \times \frac{5}{25} \times \frac{500}{L.A} \times \frac{25}{5} \times \frac{P}{100} \times 100$$

Where,

A. : Peak area of Amantadine in sample solution preparation.
B. : Average peak area of Amantadine in system suitability solution.
C. : Weight of Amantadine Hydrochloride working standard in mg.
L.A. : Labeled amount of Amantadine Hydrochloride in mg
P. : Potency of Amantadine Hydrochloride working standard on as is basis

Chromatogram

Fig.1. Chromatogram of blank

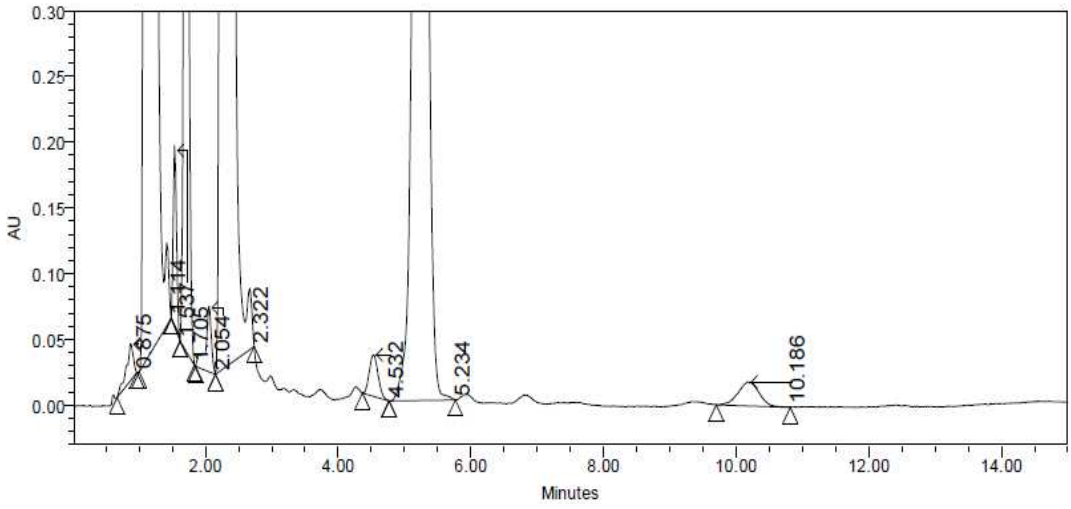


Fig.2. Chromatogram of Standard Solution

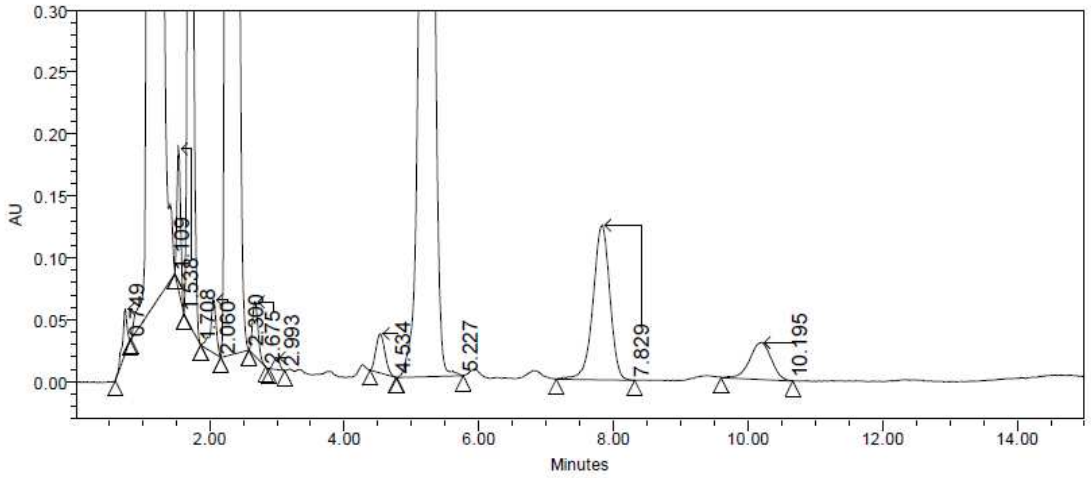
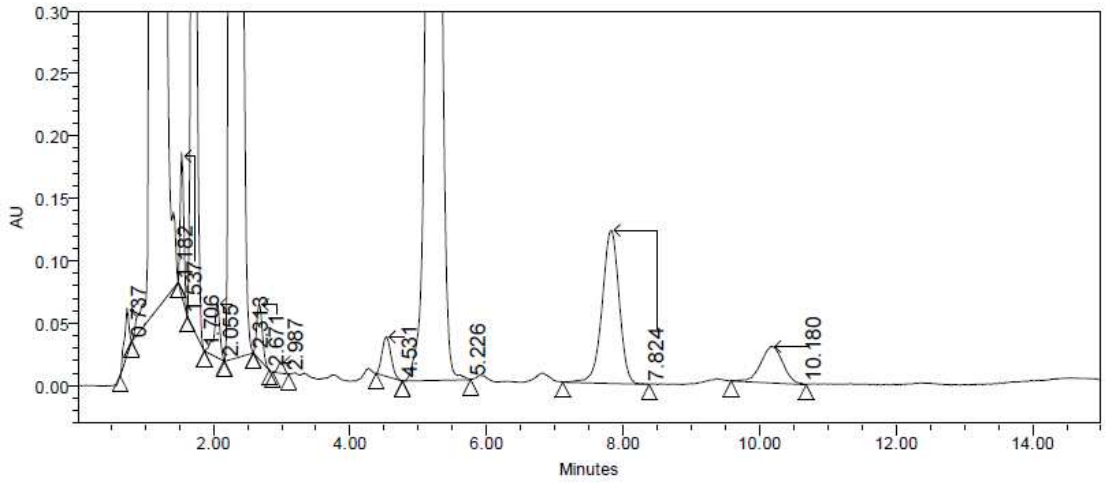


Fig.3. Chromatogram of Test Solution



Results and Discussion

Validation Results:

Sr.No	Parameters	Results
A	**System Precision**	The system suitability results are within the pre-established acceptance criteria and % RSD of peak areas of five replicate injections of system suitability solution is not more than 2.0.
B	**Method Precision**	The system suitability results are within the pre-established acceptance criteria and % RSD of the assay results of six sample solutions is not more than 10.
C	**Intermediate Precision**	The system suitability results are within the pre-established acceptance criteria and % RSD of the assay results of six sample solutions should is not more than 10. The absolute % difference between the mean result obtained in method precision and intermediate precision is not more than 10.0
D	**Linearity**	Correlation coefficient is more than 0.999
E	**Accuracy**	The system suitability results are within the pre-established acceptance criteria. Mean % recovery at each concentration is in between 95.0 to 105.0%. % RSD of %recovery at each level is not more than 5.0.
F	**Specificity(Selectivity)**	Interference of diluent blank and placebo is not observed and peak purity of Amantadine peak is passes
G	**Robustness**	The system suitability results are within the pre-established acceptance criteria

Conclusion

The Proposed method was found to be simple, accurate, economical, precise, linear and robust for estimation of Amantadine hydrochloride.

References

1) Sharma B.K.; Instrumental Method of Chemical Analysis, 21th Edition, Goel Publishing Housing, Krishna Prakashan Ltd., 2002; 3
2) Skoog, West and Holler.; Fundamental of Analytical Chemistry,7 th Edition, Saunders college Publishing, 1992; 1
3) Conners K. A.; Textbook of Pharmaceutical Analysis, 3 rd Edition, A Wiley- Intersciences Publication, 1999; 616-622.
4) Journal of applied spectroscopy, volume 73, no.6, by I.A.Darwish, A.S.Khedr, H.F.Askal, R.M.Mahmoud.
5) Analytical Chemistry by M.J.Navas and A.M.Jimenez

International Journal of Drug Discovery And Research
IJDDHR