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Validated high performance liquid chromatographic method for determination of Rasagiline Mesylate under oxidative, photolytic and thermal stress Conditions

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Abstract: The high performance liquid chromatographic method was developed for the determination of Rasagiline Mesylate in presence of its degradation products. As per the ICH guidelines Q1A (R2), forced degradation study was carried out under oxidative, photolytic and thermal stress conditions. The analysis of stressed samples was carried out using Prontosil C18 column. The mobile phase comprised of methanol and phosphate buffer (pH 3.0) in the ratio 75:25 (v/v) for 1 min and then changed to 90:10 (v/v) in next 9 min by HPLC gradient programming. The flow rate was maintained at 1.0 ml/min and detection wavelength was 210 nm using PDA detector. The validation of developed method was carried out in terms of linearity, precision, accuracy, specificity and selectivity.
Keywords: Rasagiline Mesylate, ICH, degradation.

1. INTRODUCTION

According to ICH (International Conference on Harmonization) Q1A (R2) drug stability tests guidelines [1,2] the stability-indicating assay method should be employed for the analysis of stability samples. The stress study involves the degradation behavior of drug under hydrolytic, oxidation, photolysis and thermal conditions [3]. Rasagiline Mesylate(1*R*)-2,3-Dihydro-*N*-2-propyn-1-yl-1*H*-Inden-1-amine methanesulfonate (Figure 1). It is used in the treatment of Parkinson's disease and is reversible inhibitor of MAO-B causing an increase in extracellular levels of dopamine in striatum. There are several methods reported for analysis of Rasagiline Mesylate HPLC [4,5,6,7], LC-MS [8], Spectrophotometry [9]' stability-indicating HPLC [10,11]. However the objective of the present work was to develop precise and accurate HPLC method for determination of Rasagiline Mesylate in bulk drug under ICH recommended oxidative, photolytic and thermal stress conditions. The developed method is simple and time consuming which proves the suitability of the method in comparison with the reported methods.

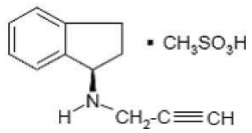

Figure 1: Structure of Rasagiline Mesylate

2. EXPERIMENTAL

2.1 Materials:

Rasagiline Mesylate was kindly procured from Dr. Reddy's Lab. The HPLC grade methanol and analytical reagent grade buffer materials and chemicals were used for the study.

2.2 Instrumentation:

The separation of drug and its degraded products were achieved using the HPLC system (Waters, Milford, USA) consisted of a 600E pump, a 996 PDA (photo-diode array) detector, Waters HPLC autosampler/Injector, Waters^{TM} 600 controller and Waters in-line degasser AF module was used. The data were acquired and processed using EMPOWER Build 1154 software. The Prontosil C18 column (150 mm × 4.6 mm i.d., with a particle size of 5 µm) column was used for the separation.

2.3 Preparation of Standard Stock and working solution:

For the preparation of standard stock solution (1000µg/ml), 25mg of drug was dissolved in 25ml of methanol and standard working solution (100µg/ml) was prepared by further dilution of stock solution with methanol.

2.4 Degradation Study:

As per the ICH recommended stress conditions, RAS bulk drug was stressed under oxidative, photolytic and thermal conditions. To study degradation under oxidative conditions, drug solution was refluxed with 30% H_2O_2 at 80^0C for 8h whereas for photostability studies, the drug powder was exposed for 24h in UV chamber. The drug powder was exposed in an oven at 60^0 for 12h for study of thermal degradation behavior of RAS. The stressed samples were collected after specific intervals and analyzed by HPLC.

3. RESULTS AND DISCUSSION

3.1 Method development and optimization by HPLC

The gradient programming was utilized to achieve the separation of RAS from its degraded products. The initial mobile phase composition was methanol and phosphate buffer (pH 3.0) in the ratio of 75:25 v/v for 1 min and then changed to 90:10 v/v in next 9 min. with a flow rate of 1.0 ml/min. The Injection volume was 50µl and detection was carried out at 210 nm. A HPLC chromatogram of Standard solution of Rasagiline Mesylate is shown in Figure 2.

3.2 Degradation behavior

The drug was found to be stable under oxidative conditions as there was no any degradation peak was seen after exposing it to 30% H202 for 8h (Figure 3). After exposing the drug powder to UV light for 24h there was absence of any major degradation peak this suggested the photostability of the RAS (Figure 4). However the drug was found to be thermally unstable after exposing the drug powder to 60^0 in an oven for 12h indicated by the presence of major degradation peak (RT DP- 4.743) (Figure 5).

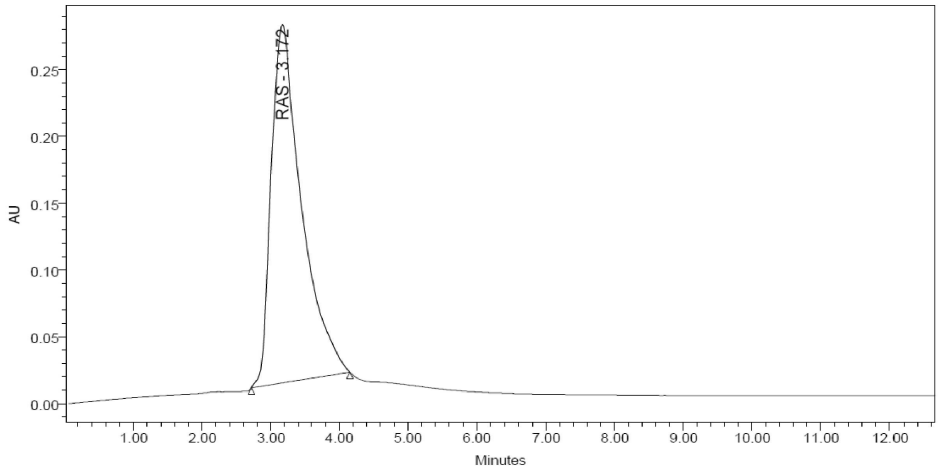

Figure 2: HPLC chromatogram of Standard solution of Rasagiline Mesylate

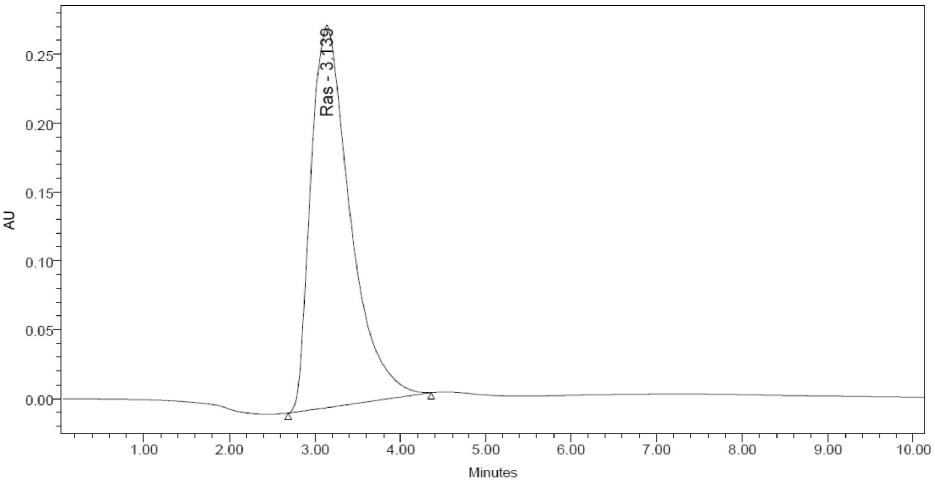

Figure 3: HPLC chromatogram of API under oxidative conditions

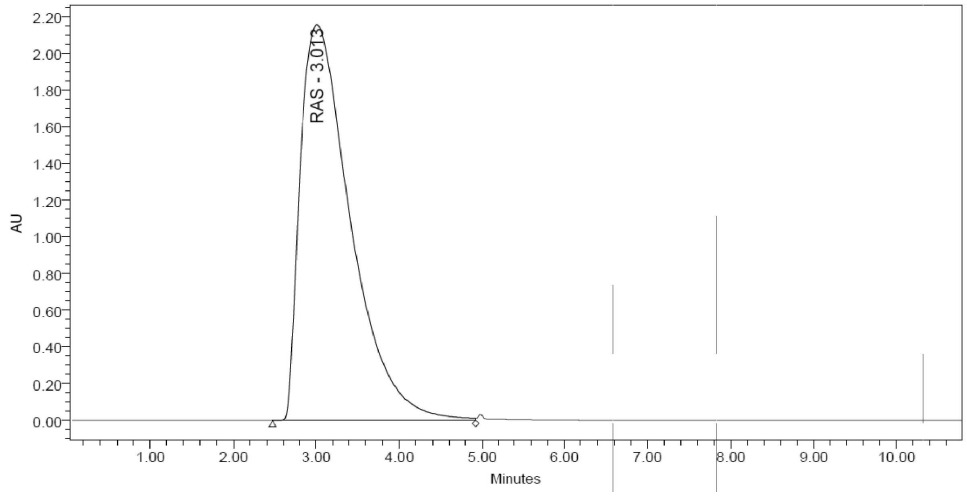

Figure 4: HPLC chromatogram of API under Photolytic conditions

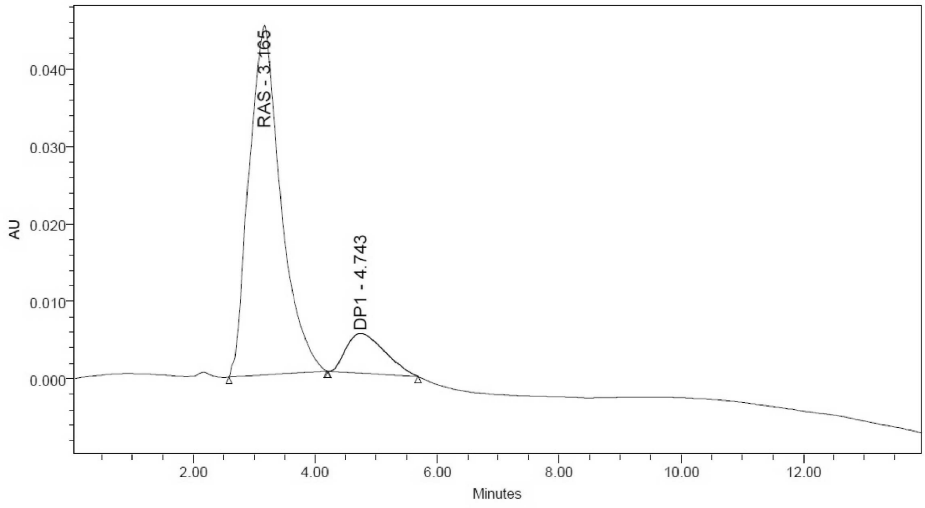

Figure 5: HPLC chromatogram of API under Thermal stress conditions

3.3 Validation

The method was validated in terms of linearity, recovery, precision, specificity and selectivity.

3.3.1 Linearity

The linearity of the method was determined by injecting five replicate injections of RAS and was found to be linear in the concentration range between 5-15µg/ml. The result of the linearity is shown in **Table 1.**

3.3.2 Recovery

The recovery study was carried out by means of standard addition method. The method was found to be accurate as good recoveries of spiked drug were obtained at each added concentration. The result of recovery experiment is shown in **Table 2.**

3.3.3 Precision

Intra-day and Inter-day precision study was carried out by analyzing three different concentrations of RAS on the same day and three different days respectively. The result of Intra and Inter-day precision is shown in Table 3.

3.3.4 Specificity and Selectivity

The method was found to be specific which was established through resolution factor of drug peak and nearest resolving peak. The selectivity of the method was established through purity plot. In purity plot of RAS in presence of its degradation products, the purity angle was found to be less than purity threshold hence method was found to be selective.

Table 1: Result of Linearity study

Concentration (µg/ml)	Mean peak area
5	616834
10	1500650
15	2566200

Table 2: Result of Recovery study (n=3)

Actual Concentration (µg/ml)	Mean Concentration Found (µg/ml), % RSD	% Recovery
8	7.82	97.75%
10	9.93	99.3%
12	11.76	98.0%

Table 3: Result of Precision study

Concentration	Intra-Day		Inter-Day	
(µg/ml)	Mean (n=3)	% RSD	Mean (n=3)	% RSD
8	7.63	1.51	8.12	1.47
10	10.2	0.98	9.83	1.55
12	11.89	1.41	11.86	1.75

CONCLUSION:

The present work reports a simple, accurate, precise and specific high performance liquid chromatographic method for determination of RAS in presence of its degradation products. The developed method is proposed for the analysis of stability samples of RAS bulk drug generated during ICH recommended stress conditions. However the method could also be extended for the analysis of pharmaceutical formulations of drug.

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Conflicts of Interest: Authors have no conflicts of interest.

REFERENCES

1. ICH (1993) Stability Testing of New Drug Substances and Products. International Conference on Harmonization, IFPMA Geneva.
2. Bakshi M Singh S (2002) Development of validated stability –indicating assay methods-critical review. J Pharm Biomed Anal 28:1011-1040.
3. ICH (2000) Stability Testing of New Drug Substances and Products (Q1AR). International Conference on Harmonization, IFPMA. Geneva.
4. Lakshmi MV, Rao S, Rao AL (2010) Development and validation of RP-HPLC method for the estimation of Rasagiline Tablet dosage form. Rasayana J Chem 3 (4):621-624.
5. Fernandez M, Negro S, Slowing K, Fernandez AC, Barcia E (2011) An effective novel delivery strategy of Rasagiline for Parkinson's disease. Int J Pharm 419: 271– 280.

6. Fernandez M, Barcia E, Negroa S (2009) Development and validation of reverse phase liquid chromatography method for the quantification of Rasagiline Mesylate in Biodegradable PLGA microspheres. J Pharm Biomed Anal 49:1185–1191.
7. Shelke P.G, Chandewar A.V. (2013) Study of Stress Degradation Behavior of Rasagiline Mesylate under Hydrolytic Conditions by High Performance Liquid Chromatography, Drug Research, DOI http://dx.doi.org/10.1055/s-0033-1355332.
8. Min Song, Li Wang (2008) Rapid and sensitive liquid chromatography–tandem mass spectrometry: Assay development, validation and application to a human pharmacokinetic study. J Chromatogr B 875:515–521.
9. Chennaiah M, Veeraiah T, Charan Singh T, Venkateshwarlu G (2011) Extractive Spectrophotometry Methods for determination of Rasagiline Mesylate in Pharmaceutical formulations using Acidic Triphenylmetahne Dyes. J Chil Chem Soc 56(4):926-929.
10. Reddy KU, Sriramulu J, Reddy UM, Reddy VP (2011) Stability –indicating RP-HPLC method for the determination of Rasagiline in pharmaceutical products. International J Sci Inno and Disc 1(1):19-32.
11. Narendra R, Rao GN,Naidu PY (2010) Stability Indicating RP-LC Method for Determination of Rasagiline Mesylate in bulk and pharmaceutical dosage forms. Int J Appl Biol Pharm Technol 1(2):247-59.

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