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Research Article

DEVELOPMENT AND VALIDATION OF HPLC METHOD FOR DETERMINATION OF TRACE LEVEL POTENTIAL GENOTOXIC CHLORO IMPURITY IN RASAGILINE MESYLATE

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ABSTRACT

A rapid, sensitive and selective High performance liquid chromatography (HPLC) method was developed and validated for the trace analysis of N-(2-Chloroallyl)-1-(R)-aminoindan, a potential genotoxic impurity in Rasagiline mesylate drug substances. HPLC column used was Sunfire C18 (250 mm x 4.6 mm, 5 µm). The gradient elution mode was selected where mobile phase-A consist of Buffer (40Mm Ammonium dihydrogen phosphate solution adjusted to pH 7.0 with orthophosphoric acid) and mobile phase-B in 60:40 ratio, whereas mobile phase B consist of solvent Acetonitrile and Methanol in the ratio 70:30. The flow rate was set to 1.5 mL/min and run time of 35 minutes. UV detector with wavelength 210 nm was used for the analysis. Column oven temperature was kept 40°C. The developed method was validated according ICH guideline and found to be linear in the range of 0.51 ppm to 1.53 ppm for N-(2-Chloroallyl)-1-(R)-aminoindan (Chloro impurity) with a correlation coefficient 0.9973. Limit of detection and limit of quantitation was found to be 0.08 ppm and 0.23 ppm respectively. Recovery for this impurity was found between 92.75% and 102.49%. Method was found to be specific, selective, precise, and robust. The developed method can successfully be applied for the determination of Chloro impurity in Rasagiline Mesylate upto very low trace level concnetration.

KEYWORDS: *Rasagiline Mesylate, Genotoxic, Chloro Impurity, Development and Validation.*

INTRODUCTION

Rasagiline Mesylate is chemically known as (R)-N-(prop-2-yn-1-yl)-2,3-dihydro-1H-inden-1-amine methanesulfonate (Figure-1). Rasagiline is an irreversible monoamine oxidase inhibitor used in the treatment of Parkinson's disease [1, 2]. N-(2-Chloroallyl)-1-(R)-aminoindan is a potential gentotoxic process impurity that may be present in Rasagiline Mesylate. Many of the guidelines and pharmacopoeia raise the concern to limit the potential genotoxic impurities (PGIs) in active pharmaceutical ingredients (APIs) to safety level which is the threshold of toxicological concern (TTC). Genotoxic impurities could cause DNA damage involving genetic mutations. A list of such alerting functional groups have been compiled on the basis of the structure of known genotoxic compounds and their mechanism of action. If impurities bearing one or more alert function group have to be considered as potential genotoxic compounds if no toxicological data are available, and their limit has to be calculated according to specific guidelines. One such category of genotoxic impurities is chloroallyl, these are universally regarded by regulatory authorities as genotoxic potential impurities in drug substance may be formed during the synthesis of Rasagiline Mesylate [3, 4].

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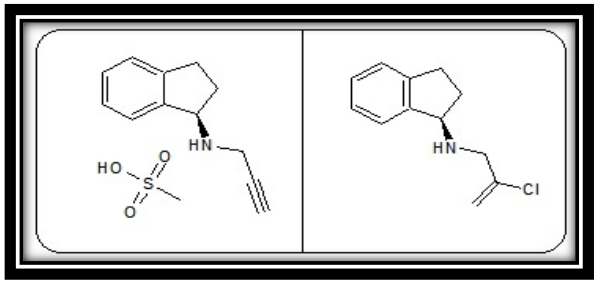

Fig. 1: Structure of Rasagiline mesylate and chloro impurity

Hence, in order to meet the regulatory requirements, it is essential to develop a highly sensitive analytical method that can identify and determine Chloro impurity in Rasagiline Mesylate at trace level. The threshold of toxicological concern (TTC) value of 1.5 µg/day intake of genotoxic impurity is permitted as per the regulatory guideline. The concentration limit in ppm of genotoxic impurity in drug substance is a ratio of TTC in µg/day intake and daily dose in g/day. Since 1 mg of Rasagiline Mesylate is administered per day [5-7], therefore, the permissible limit for genotoxic impurities comes out to be 1500 ppm/day. In the current experiment, the limit set in Rasagiline Mesylate is 1.0 ppm.

EXPERIMENTAL

Material:

Rasagiline Mesylate bulk drug sample and Chloro impurity was provided by Analytical research and development department of Indoco research Centre, Navi Mumbai. HPLC gradient grade acetonitrile and methanol were purchased from Merck chemicals. Di-ammonium Hydrogen phosphate and orthophosphoric acid were purchased from Merck chemicals while water used for preparations of solution was from Milli-Q.

Instrumentation:

Waters, Alliance 2695 series HPLC system (Milford) comprising a quaternary pump, an autosampler, a thermostatted column compartment, a solvent cabinet with degasser along with photodiode array (PDA) 2998 and ultraviolet (UV) 2487 detectors were used for separation and detection. Data acquisition and calculations were carried out using Waters Empower3 software (Milford). Sartorius (Germany) analytical balance was used for weighing of the materials.

Methodology:

Chromatographic condition:

This novel method was developed using Sunfire C18 HPLC column having length 250 mm and internal diameter of 4.6 mm, which is packed with 5 µm particle size. Separation was achieved by gradient elution mode (Table-1) by using Mobile phase-A and Mobile phase-B with a flow rate of 1.5 mL/min and injection volume of 25µL. The column temperature was maintained at 40°C ± 2°C and the peaks were monitored at wavelength 210 nm.

Table No. 1: Gradient elution

Time (min)	Mobile phase-A (%)	Mobile phase-B (%)
0	100	0
4	100	0
10	85	15
12	80	20
15	80	20
20	50	50
25	50	50
30	100	0
35	100	0

Preparation of Mobile phase:

Buffer:

Transfer about 5.4 g of Di-ammonium hydrogen phosphate into 1L bottle, dissolve in 500 mL of Water, add 500 mL Water and mix well. And adjust the pH of solution to 7.0 ± 0.05 with Ortho-phosphoric acid. Filter the solution through a 0.45µm membrane, and degas by sonication for 5 minutes.

Mobile Phase-A:

Mix 600 volumes of Buffer and 400 volumes of Mobile phase-B and degas by sonication for 5 minutes.

Mobile Phase-B:

Prepared by mixing acetonitrile and methanol in ratio of 70:30 (v/v)

Preparation of diluent:

Prepared by mixing water, acetonitrile and methanol in ratio of 70:20:10 (v/v/v) respectively.

Preparation of Blank:

Transfer 2.0 mL of 0.1 N sodium hydroxide solution to 10 mL volumetric flask and make upto mark with diluent.

Standard stock solution:

Transfer 10.0 mg of N-(2-Chloroallyl)-1-(R)-aminoindan standard into 100 mL volumetric flask, dissolve in about 20 mL of diluent and make upto mark with diluent. Transfer 5.0 mL of this solution to 100 mL volumetric flask and make upto mark with diluent.

Standard solution (1.0 ppm % w.r.t. sample):

Transfer 0.5 mL of Standard stock solution to 25 mL volumetric flask, add 5.0 mL of 0.1 N Sodium hydroxide solution into it, mix and make upto mark with diluent.

Preparation of Test sample solution:

Transfer 1000.0 mg of Rasagiline Mesylate test sample into a 10 mL volumetric flask. Add 2.0 mL of 0.1 N Sodium hydroxide solution into it. Dissolve and make upto mark with diluent.

RESULTS AND DISCUSSION

Analytical method validation:

The analytical method validation work is conducted according to the International Conference on Harmonization (ICH) guidelines. The parameter with which analytical method is validated is Specificity, Limit of detection, Limit of quantitation, Linearity, Accuracy, Precision, Robustness and Solution stability [8-11].

Specificity:

As specificity is the capability of the method to measure the analyte response in presence of its potential impurities. Chloro impurity was spiked in test sample at its limit level and analysed. Rasagiline and Chloro impurity were well separated from each other in spiked test sample (Figure-2, Table-2). There was no interference from peaks due to blank and test sample peaks. Peak purity of Rasagiline and Chloro impurity were passing for spiked test sample solution (Table-3).

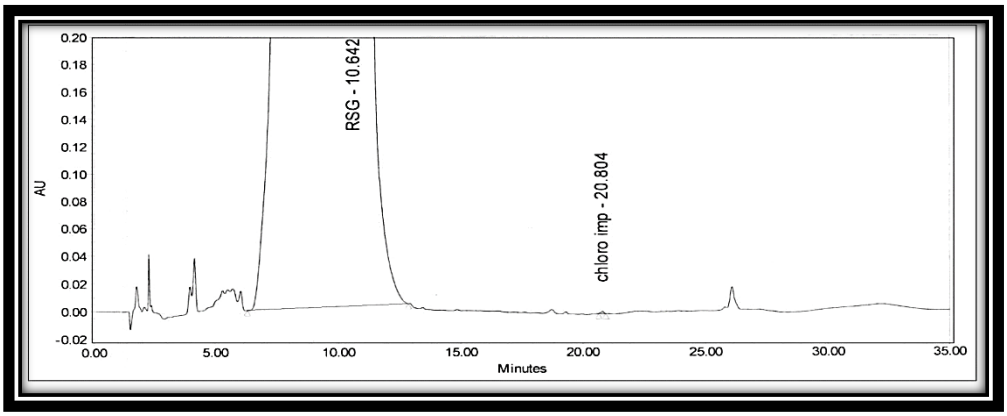

Fig. 2: Rasagiline Mesylate spiked test sample chromatogram

Table No. 2: RRT of Rasagiline and Chloro impurity

Component	Retention time (minutes)	Relative retention time
Rasagiline	10.6	1.00
Chloro impurity	20.8	1.95

Table No. 3: Peak purity data

Peak name	Purity angle	Purity threshold	Peak purity
Rasagiline (RSG)	0.451	0.925	Pass
Chloro impurity	0.091	0.654	Pass

Limit of detection and quantitation:

Series of standard solutions of Chloro impurity was prepared in concentration ranging from 50% to 150% of target concentration (1 ppm w.r.t. sample). Limit of detection (LOD) and Limit of quantitation (LOQ) was calculated based on residual standard deviation of regression line and slope. Limit of detection obtained was 0.08 ppm and Limit of quantitation 0.23 ppm.

Linearity:

Series of linearity solution of Chloro impurity were prepared from 50 to 150% of target concentration (1.0 ppm w.r.t. sample). Linearity curves were drawn by plotting the peak area of Chloro impurity against its corresponding concentration of linearity solution. Regression coefficient and % y intercept are reported (Figure-3). Regression coefficient observed was 0.9973 and % y-intercept 2.27.

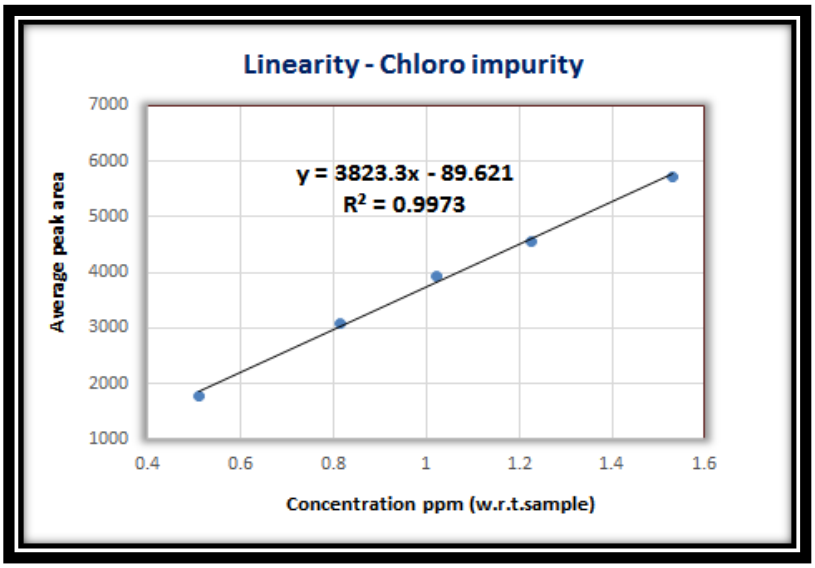

Fig. 3: Linearity graph of Chloro impurity

Precision:

System precision was carried out by analysing six standard solution of Chloro impurity at limit level concentration (1.0 ppm). Relative standard deviation for peak area of Chloro impurity was calculated and found to be 2.54 %. Precision at LOQ solution was prepared at LOQ concentration of Chloro impurity and injected six times. Relative standard deviation for peak area for Chloro impurity obtained was 8.36. For repeatability and intermediate precision, six solutions were prepared by spiking the Chloro impurity in test sample at limit level concentration (1.0 ppm). Relative standard deviation observed for spiked Chloro impurity content in repeatability and intermediate precision solution was 2.02 % and 2.05 % and cumulative of repeatability and intermediate precision was 2.15 %.

Accuracy:

The accuracy of the method was established by performing the recovery studies of Chloro impurity, which was spiked at 50%, 100% and 150% in Rasagiline Mesylate test sample in triplicate and analysed for its recovery. Recovery for Chloro impurity obtained was between 80% and 100%.

Robustness:

For robustness, four deliberate changes were done with respect to flow rate and column oven temperature. Each change consists of one upper set and one lower set (Table-4). For each set, three preparations were done by spiking the Chloro impurity in the test sample at the limit level and analysed. Relative standard deviation for spiked Chloro impurity content observed was less than 5.0 %. Cumulative relative standard deviation of robustness and repeatability determination was less than 10 % (Table-4).

Table No. 4: Robustness parameter changes

Sr. No.	Changes	Relative standard deviation	Cumulative Relative standard deviation
1	Mobile phase flow rate 1.4 mL/min	2.46 %	2.49 %
2	Mobile phase flow rate 1.6 mL/min	3.29 %	5.24 %
3	Column Oven Temperature by 35°C	3.02 %	3.36 %
4	Column Oven Temperature by 45°C	2.86 %	3.70 %

Solution stability:

Test solution stability was established by injecting the same test sample solution kept at room temperature after every six hour time interval for 24 hours (Table-5). Chloro impurity content in test sample solution for all determination was calculated and relative standard deviation for impurity content was found out to be less than 5.0 %, thus solution stability was established up to 24 hours.

Table No. 5: Solution stability of Rasagiline Mesylate

Time interval	Chloro impurity content (ppm w.r.t. sample)
0 Hr	Below Detection Level
6 Hrs	Below Detection Level
12 Hrs	Below Detection Level
18 Hrs	Below Detection Level
24 Hrs	Below Detection Level
% Relative standard deviation	0.0

CONCLUSION

The reverse phase HPLC method is developed for quantitative determination of Chloro impurity of Rasagiline Mesylate. This method is validated and found out to be linear, accurate, precise, robust and specific. Acceptable data for all method validation parameters tested and found out to be satisfactory. The developed method can suitably use by quality control department to determine the genotoxic Chloro impurity in commercial and stability test samples of Rasagiline Mesylate.

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