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Development and validation of a new HPLC method with fluorescence detection for determination of Rasagiline Mesylate and its application to pharmaceutical dosage form

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ABSTRACT

A reverse phase high performance liquid chromatographic method was developed and validated for the determination of Rasagiline Mesylate in the dosage form. Chromatographic separation was carried out on an RP-18 column using a mobile phase consisting of (acetonitrile: 0.02 M ammonium acetate (60:40 v/v). The flow rate was 1.0ml/min. Fluorescence detector was employed with excitation at 210nm and emission at 288 nm. The calibration curve was linear over the range (0.5–3.00) µg/ml with a correlation coefficient of 0.998. The simplicity and rapidity of the developed method made it very suitable for routine analysis of Rasagiline Mesylate in the tablet dosage form.

Introduction

Parkinson disease is recognized as one of the most common neurologic disorders, affecting about 1% of individuals older than 60 years [1]. The most important and debilitating symptoms of Parkinson's disease are those resulting from dopamine (DA) depletion in the nigrostriatal pathway [2]. The neurochemical basis of Parkinson disease involves loss of Dopamine level resulting from oxidation with monoamine oxidase B which exists in corpus striatum of the brain. Rasagiline (N-propargyl-1-(R)-Aminoindan) is a novel, highly potent irreversible monoamine oxidase (MAO)-B inhibitor, anti-Parkinsonian drugs [3.4]. Rasagiline inhibits monoamine oxidase activity resulting in increasing the level of Dopamine in the brain [5].

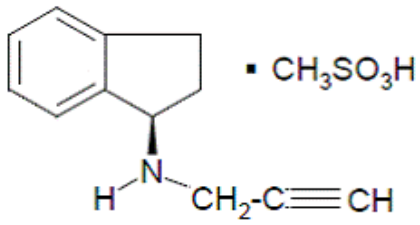

Fig. 1: Chemical structure of Rasagiline Mesylate [6]

There are some reported methods in the literature for quantification of Rasagiline Mesylate. For example- Spectrophotometric methods [7,8] to asses Rasagiline in bulk with ranges 25 – 300 µg/ml and 39–273 µg/mL, respectively. Also High performance liquid chromatography methods to determine Rasagiline in pharmaceutical dosage forms were reported [8,9] with ranges 39–117 µg/mL, 0.5 – 20 µg/mL. Moreover high-performance liquid chromatography with ultraviolet detection was developed for estimation of process–related impurities and forced degradation products of Rasagiline Mesylate in pharmaceutical formulation [10]. In addition, an HPLC method for determination of Rasagiline Mesylate in plasma was reported [11]. Stability-Indicating HPTLC method was used for analysis of Rasagiline Mesylate in the bulk drug and tablet dosage form [12], LC–MS/MS methods for determination Rasagiline in plasma were also reported [13,14].

The aim of this study was to develop a new fluorescence detection method with high-performance liquid chromatography to determine Rasagiline in tablet dosage form with high sensitivity and its application in dissolution study for comparison between test and reference listed drug in vitro.

Materials and methods

Instrumentation

A High-Performance Liquid Chromatography (HPLC) was employed in this study consisted of a solvent delivery system (SHIMADZU LC-20AT, Japan) isocratic pump, Shimadzu Autosampler (Model SIL-20A, Japan), a Spectrofluorometric detector (SHIMADZU Detector model RF-10Axl, Japan)

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equipped with a Xenon lamp and an automatically controlled, 200-650 (nm), a 12µl square quartz flow cell, Dissolution tester (Hanson vision ELITE 8, USA), Spectrophotometer (Shimadzu UV-1650pc UV-Vis Spectrophotometer, Japan) and Balance (Shimadzu AUY 220, Japan).

Reagents and chemicals

Rasagiline mesylate reference standard was from Lupin Limited (Mumbai, India). Parkintreat 1 mg Tablets from Inspire Pharmaceutical Company (Cairo, Egypt). Azilect® 1 mg Tablets from Teva Pharmaceuticals (USA), HPLC grade water and hydrochloric acid are from Scharlau (Barcelona, Spain), Methanol and acetonitrile HPLC grade from Fischer scientific (Loughborough, United Kingdom), ammonium acetate from El Nasr pharmaceutical chemicals company (Qaliubiya, Egypt)

Selection of excitation and emission wavelengths of the method

Standard solution of 2µg/ml Rasagiline was scanned between 200nm to 400nm with UV spectrophotometer. The maximum absorbance was found at 210nm. The same procedures were repeated using Fluorescence spectrophotometer scanner and the maximum emission wavelength was found to be 288 nm.

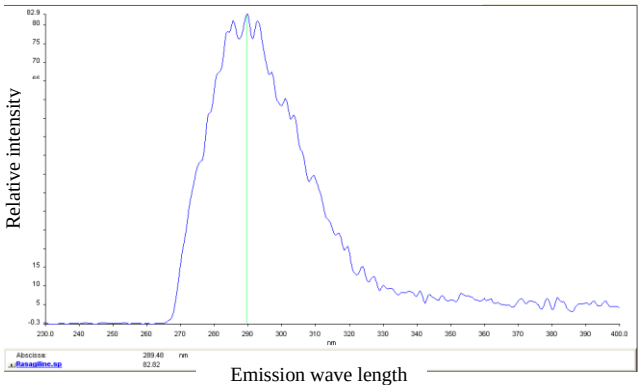

Fig. 2: Emission spectra of Rasagiline Mesylate

Chromatographic conditions

Isocratic elution of the mobile phase 0.02 M Ammonium Acetate in water: Acetonitrile (40:60 v/v) with the flow rate of 1 ml/min. The contents of the mobile phase were filtered and degassed by sonication before use. Separation was performed on (Discovery C18 (Supleco) (150 x 4.6) mm, 5µm particle size) as a column. The analysis was performed using the Shimadzu SpectroFluorometric detector to determine the peak area. The total run time was set at 6 min. The injection volume was 100 µL. The wavelength used was at 210 nm Excitation, and 288 nm Emission. The developed method was validated in terms of accuracy, linearity, specificity, intra-day and inter-day precision and robustness for the assay of Rasagiline as per FDA guidelines.

Standards and sample solutions preparation

A stock solution of 400 µg/ml Rasagiline was prepared in methanol/water mixture 1:1, then 1.00 ml of this solution was completed to 10 ml with methanol/water mixture 1:1 to give a final concentration of 40 µg/ml.

Assay of rasagiline mesylate from its tablet dosage form

Twenty tablets (Parkintreat 1 mg Tablets Inspire Pharmaceutical Company, Egypt) were weighed and finely powdered. A precisely weighed portion of the powder equivalent to 1 mg of Rasagiline was extracted with methanol by the aid of sonication for five minutes. The extract was transferred to a 50 ml volumetric flask and made up to the mark with the Methanol. The solution was filtered through a 0.45 µm membrane filter. The tablet extract was appropriately diluted with mobile phase to obtain a concentration of 2 µg/mL.

Validation of the developed method

The developed method was validated in terms of accuracy, linearity, specificity, intra-day and inter-day precision and robustness for the assay of Rasagiline as per FDA guidelines [15].

1. **Specificity**
   The specificity of the method was investigated by analyzing the drug product comparing with Rasagiline standard solution using the same chromatographic conditions defined for the method. It was found that there is no interference due to excipients in the tablet formulation.
2. **Linearity and range**
   Linearity with range of (0.5 to 3.00) µg/ml was prepared by taking the volumes of (125, 250, 375, 500, 625, 750) µl from 40 µg/ml into 10 ml flasks and complete with 0.1 N hydrochloric acid to get the following concentrations (0.5, 1.00, 1.50, 2.00, 2.50, 3.00) µg/ml.
   The linearity of Rasagiline was determined by plotting peak area versus concentrations.
3. **Accuracy and Precision**
   The accuracy of the method was established by analyzing three different concentrations of Rasagiline (0.50, 1.00, 2.50µg/mL). The precision of the method was established by injecting six replicate standard samples for the concentration (2 µg/mL) and the intermediate precision was determined by analyzing standard samples on two different days. Accuracy was expressed by evaluating the amount determined from the quality control standards and comparing to the respective nominal value expressed as percent recovery. Precision was expressed as the relative standard deviation of the analyte response.
4. **Robustness**
   To determine the robustness of the method, experimental conditions such as pH of mobile phase and flow rate were slightly altered. The effect of small changes in chromatographic conditions was studied to verify the robustness of the method.

Dissolution Test

The dissolution tester consisted of a water bath maintained at 37^{o}C, eight vessels containing 500 ml of 0.1 N hydrochloric acid solution as the dissolution medium which simulate the stomach. The apparatus used for stirring the drug and the medium of dissolution is

called paddle or USP apparatus 2 that was set at 50 rpm (rotation per minute) [16].

Twelve tablets of each test and reference were tested after collection of 1 mL of each vessel at 10, 15, 30 and 45 min and a fresh dissolution medium of 1 mL was added after each collection of samples to keep the volume of dissolution constant. Samples were transferred from the vessels into vials after being filtered through a nylon filter (0.45 µm) to be injected HPLC.

Results and discussion

Chromatography optimization

Different chromatographic conditions were optimized. The Fluorescence detector was set at 210 nm excitation and 288 for emission, different organic modifiers with different ratios, and different strengths of the buffer were tried also, different pH values for mobile phase, different types of stationary phases were tried to achieve the best separation within a short analysis time with the highest sensitivity.

Validation

1. **Specificity**
   Specificity of the method was proved by the absence of interfering peaks from excipients present in formulations. Chromatograms obtained from tablets were almost identical to those obtained from the standard solutions.
2. **Linearity and range**
   The linearity of the method was established over the analytical range of 0.50-3.00 µg/ml (**Fig. 4**). Good correlation between analyte peak area and concentration of the drug was obtained with correlation coefficient (r2) = 0.998
3. **Accuracy and Precision**
   Recovery experiments were employed to determine the accuracy of the proposed analytical method. Three different concentrations were prepared in triplicate (25, 75 and 125 %) of 2 µg/ml to be analyzed. The same three concentrations were compared to the standard solution. The values of recovery for injected samples showed high recovery values (100.39, 99.47 and 99.85 %, respectively). The recovery percentage results of the method are given in **Table 1**.
   The results of the precision study are given in **Table 2**. The repeatability (within-day precision) expressed as RSD was 0.506 %. The intermediate precision (day-to-day) precision was up to 0.902 %
4. **Robustness**
   The robustness of the method was studied by small changes in the method such as altering the mobile phase pH, flow rate; sample solution of 2 µg/ml was injected in triplicate and the peak area RSD and were evaluated. It was observed that there were no changes in the chromatograms. The results are given in **Table 3**.

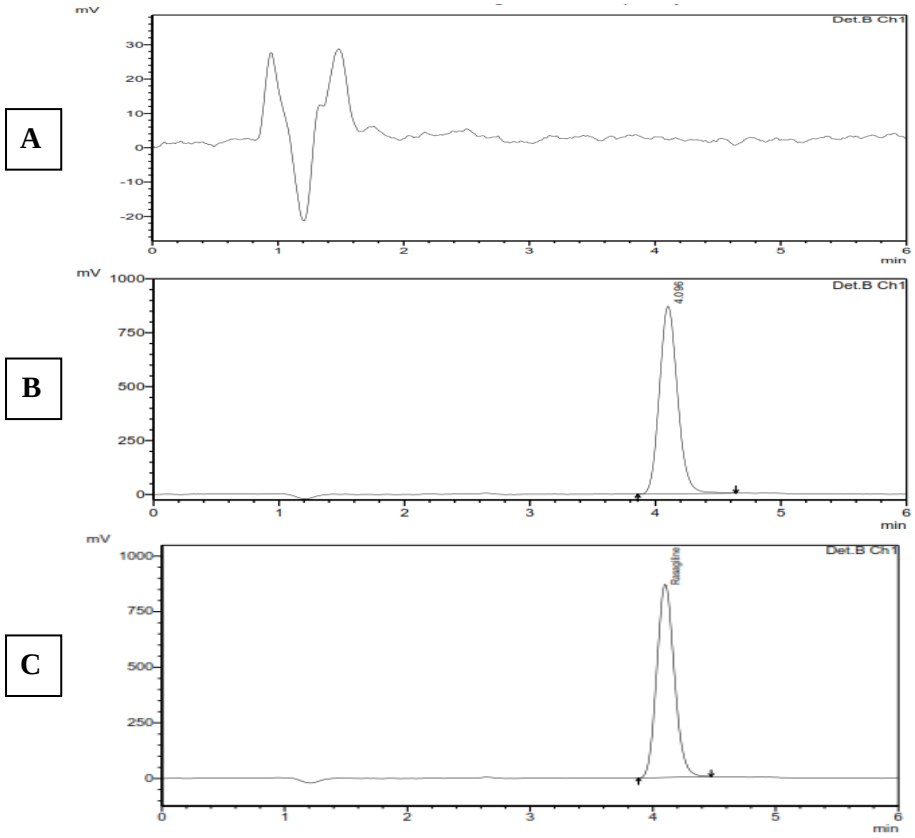

Fig. 3: Representative fluorescence chromatograms of Rasagiline Mesylate **(A)** chromatogram of blank, **(B)** chromatogram of (2 µg/ml) Rasagiline Mesylate in mobile phase. **(C)** Chromatogram of (2 µg/ml) Rasagiline Mesylate in tablet extract.

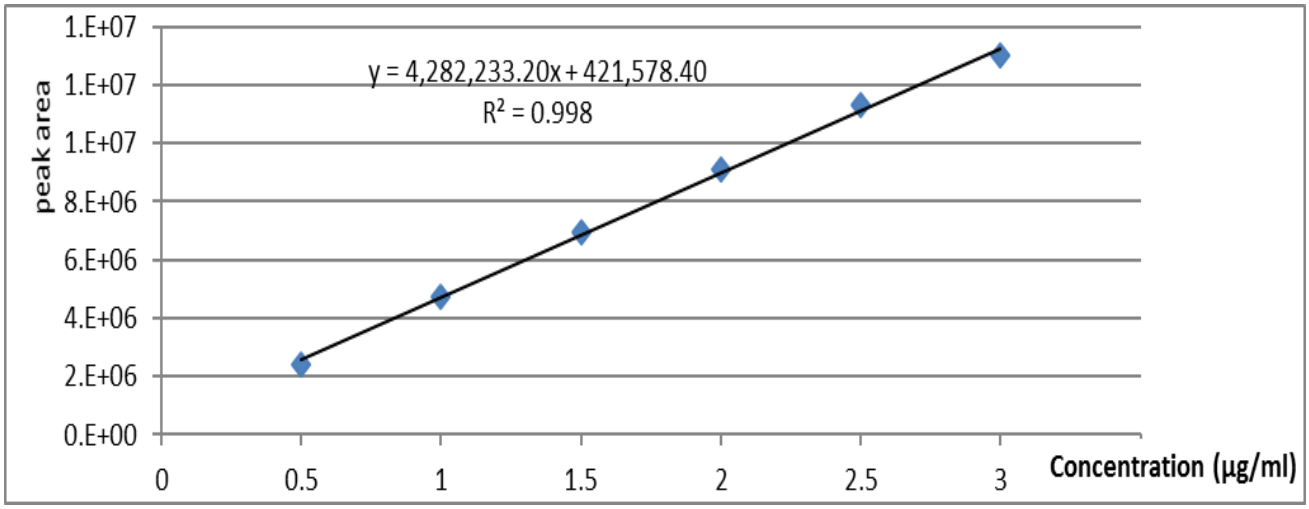

Fig. 4: Linearity range of Rasagiline Mesylate from (0.5 -3.00 µg/ml).

Table (1): Results of the Recovery Tests for Rasagiline Mesylate .

Sample number	Concentration in µg/ml	Standard response	Response	% Recovery	Recovery mean	Recovery RSD%
1	0.5	2409844	2451746	101.74	100.39	1.20
			2409727	100.00		
			2395907	99.42		
2	1.5	6939270	6892583	99.33	99.47	0.13
			6907477	99.54		
			6908019	99.55		
3	2.5	11339689	11241382	99.13	99.85	0.62
			11362734	100.20		
			11362869	100.20		

Table (2): Intraday and interday precision of the method .

Injection number	Concentration	Intraday precision	Interday precision
1	2 µg/ml	9054573	9959799
2		9073282	9733129
3		9055407	9944919
4		9007869	9976117
5		9064666	9874430
6		9149002	9898342
Mean		9067467	9897789
SD		45941.78	89286.75
RSD %		0.51	0.90

Table (3): Robustness of the method.

Parameter	Variance	Concentration	Mean peak area	RSD n=3
Flow rate	1ml/min	2 µg/ml	9787430	0.40
	1.1ml/min		10054888	0.85
pH of mobile phase	pH= 7.25		9822276	0.89
	pH= 7.50		10360914	1.02

Dissolution test

The method was applied for the analysis of Rasagiline Mesylate in comparison of Parkintreat 1 mg Tablets as the generic Product against Azilect® 1 mg Tablets As the Reference product.

Calculations:

y = 5080997.8286x – 29970.2

Y= Peak Area

X= concentration µg/ml

$$\%Dissolution = \frac{\text{concentration } (\mu g/ml) * 500 * 100}{1 * 1000}$$

Dissolution results

Table (4): Cumulative % Dissolution Data of Rasagiline from Azilect® 1 mg Tablets (Teva Pharmaceuticals, USA), the Reference product after (minutes).

EXP No.	*Cumulative % Dissolution of Data of Rasagiline from Azilect® 1 mg Tablets (Teva Pharmaceuticals, USA), after (minutes)*			
	10	**15**	**30**	**45**
I	91.36	91.29	86.91	87.82
II	95.46	92.04	91.99	87.40
III	91.88	89.42	95.70	87.10
IV	90.99	90.59	92.19	90.94
V	88.54	88.06	91.19	89.70
VI	92.65	89.77	94.78	89.71
VII	90.46	88.45	97.79	89.56
VIII	90.65	89.28	94.57	89.52
IX	88.41	88.18	92.23	89.09
X	93.90	88.45	97.04	88.73
XI	91.96	87.11	98.14	89.08
XII	93.01	89.77	92.59	91.93
Average	**91.61**	**89.37**	**93.76**	**89.21**

Similarity and Difference Factors

The calculated Difference factor between the dissolution profile of Rasagiline from Parkintreat 1 mg Tablets (SEDICO For Inspire Pharmaceutical Co. (IPC Pharma), Egypt) and Azilect® 1 mg Tablets (Teva Pharmaceuticals, USA) = 7

$$f_1 = \left\{\left[\sum_{t=1}^{n} | R_t - T_t \right] / \left[\sum_{t=1}^{n} R_t \right]\right\} .100$$

According to the FDA Guidance for Industry, for dissolution profiles to be considered similar, the difference factor should be close to 0. Generally, the difference factor values up to 15 (0-15) ensure sameness or equivalence of the two profile curves and, thus, of the performance of the test and reference products.

The calculated Similarity factor between the dissolution profile of Rasagiline from Parkintreat 1 mg Tablets (SEDICO For Inspire Pharmaceutical Co. (IPC Pharma), Egypt) and Azilect® 1 mg Tablets (Teva Pharmaceuticals, USA) = 60

$$f_2 = 50 \cdot \log\left\{\left[1 + (1/n)\sum_{t=1}^{n}(R_t - T_t)^2\right]^{-0.5} \cdot 100\right\}$$

According to the FDA Guidance for Industry, for dissolution profiles to be considered similar, the similarity factor should be greater than 50 (50-100) ensure sameness or equivalence of the two profile curves and, thus, of the performance of the test and reference products [17].

Table (5): Cumulative % Dissolution Data of Rasagiline from Parkintreat 1 mg Tablets (SEDICO For Inspire Pharmaceutical Co. (IPC Pharma), Egypt), the Test product after (minutes).

EXP No.	*Cumulative % Dissolution of Data of Rasagiline from Parkintreat 1 mg Tablets (SEDICO For Inspire Pharmaceutical Co. (IPC Pharma), Egypt), after (minutes)*			
	10	**15**	**30**	**45**
I	87.61	99.22	94.10	95.85
II	88.25	97.47	95.45	94.65
III	91.37	96.00	98.63	94.87
IV	81.61	95.75	101.73	97.12
V	84.59	94.42	99.21	96.80
VI	85.86	93.33	100.25	96.62
VII	89.35	95.03	101.11	97.79
VIII	80.52	94.33	103.91	96.38
IX	83.82	93.08	101.13	95.44
X	86.32	93.50	102.10	95.20
XI	90.03	93.09	102.98	96.10
XII	81.63	93.84	103.37	94.36
Average	**85.91**	**94.92**	**100.33**	**95.93**

Limits: Not less than 80% dissolved after 45 minutes.

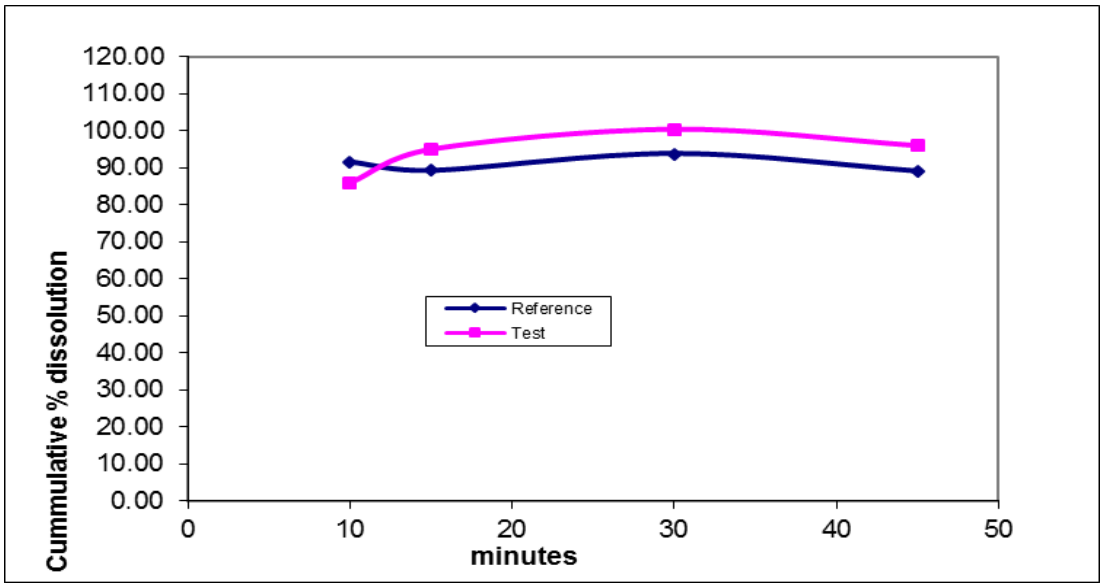

Fig. 5: Dissolution Profile of Rasagiline from Parkintreat 1 mg Tablets (SEDICO for Inspire Pharmaceutical Co. (IPC Pharma), Egypt), the test product and Azilect® 1 mg Tablets (Teva Pharmaceuticals, USA), the reference product.

Conclusion

A new method using fluorescence detection method has been developed for estimation of Rasagiline Mesylate in the tablet dosage form. The proposed method is simple, accurate, sensitive, and linear across the specified range. So that, it is suitable for the determination of Rasagiline Mesylate in tablet dosage form.

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