Research Article

VALIDATED AND STABILITY INDICATING DISSOLUTION TEST WITH REVERSE PHASE-HPLC ANALYSIS FOR RASAGILINE MESYLATE IN TABLET DOSAGE FORM

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ABSTRACT

Rasagiline is a highly potent, selective and irreversible second-generation monoamine oxidase inhibitor with selectivity for type B of the enzyme (MAO-B). The present studies aimed at developing and validating a rapid and sensitive high performance liquid chromatography method for dissolution test of rasagiline mesylate tablets. The appropriate conditions were determinate after testing sink conditions; dissolution medium, agitation intensity and the USP apparatus, paddle was applied. The best dissolution conditions tested for the products in each respective pharmaceutical dosage form were applied to evaluate the dissolution profiles. In order to choose the most discriminatory one, the conditions to carry out the dissolution tests were 500 mL of 0.1 M HCl as dissolution medium, paddle at 50 rotations per minute (rpm) stirring speed for tablets, time test set at 45 minutes and detection was carried out at 210 nm. The retention time of rasagiline mesylate was found to be 4.15 minutes. The developed and validated dissolution tests satisfactorily describes the time-course of the drug release. The HPLC method was validated to meet requirements for a global regulatory filing and also to quantify rasagiline mesylate tablets from the dissolution tests.

Keywords: Parkinson's disease, Rasagiline mesylate, RPLC, Solubility, Stability, Validation.

INTRODUCTION

In recent years, more emphasis has been placed on dissolution testing within the pharmaceutical industry and by regulatory authorities.[1] The dissolution tests for immediate release solid oral dosage forms, such as tablets, are used to assess lot-to-lot quality of a drug product; guide development of new formulations and ensure continuing product quality and performance after certain changes, such as changes in the formulation, and the manufacturing process.[2] From a biopharmaceutics point of view, a more discriminating dissolution method is preferred because the test will indicate possible changes in the quality of the product before in vivo performance is affected.[3]
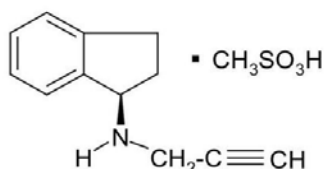

Figure 1: Chemical structure of rasagiline mesylate

Rasagiline mesylate [N-propargyl-1 R(+)-aminoindan] is a highly potent, selective, irreversible, second-generation monoamine oxidase inhibitor with selectivity for type B of the enzyme (MAOB) [Figure 1],[4] which has been evaluated for the treatment of Parkinson's disease. Rasagiline also possesses neuroprotective properties that are independent of its MAO inhibitory activity.[5, 6] Preclinical studies have demonstrated that propargylamines can protect neurons from a variety of

toxins in both in vitro and in vivo models.[7] Unlike selegiline, rasagiline has no amphetamine-like metabolites and its major metabolite, 1-(R)-aminoindan, has demonstrated favourable pharmacological activity in experimental studies.[8, 9] Rasagiline was generally well tolerated in clinical trials as both monotherapy and when administered with other anti-parkinsonian drugs.[10]

A few HPLC[11] and LC-MS/MS[12, 13] methods were reported earlier for the determination of rasagiline in bulk and pharmaceutical dosage forms. There is no dissolution test described in literature for rasagiline mesylate in pharmaceutical dosage forms. Dissolution test has emerged in the pharmaceutical field as a very important tool to characterize drug product performance. Therefore the dissolution studies are used not only to assess batch-to-batch consistency of drug release from solid dosage forms, but they are also essential in several stages of formulation development, for screening and proper assessment of different formulations. To assist in regulatory filing, product quality control, and dose regimen design for clinical trials, characterization of human pharmacokinetics is generally the first and foremost clinical investigation of new drugs Moreover, the in vitro dissolution studies are relevant to predict in vivo performance of a drug release and have been used as a tool to estimate bioavailability of the drug.[14, 15] Parameters to set up the dissolution test should be researched and defined for drugs that do not possess official monographs.[16] For this reason, there is a crescent number of works describing the development of dissolution test for nimodipine, diacerhein and deflazacort.[17, 18] Having said that, the aim of the present

study was to develop a rapid and sensitive HPLC method for dissolution test of rasagiline in tablet forms, and utilize the method, after its full validation to characterize drug release.

MATERIALS AND METHODS

Materials and reagents

Rasagiline mesylate reference substance (Batch no: RMXP100001, Potency: 99.2%) and pharmaceutical formulations containing rasagiline mesylate 1 mg (Batch no: RAS-T-0016) were obtained from Orchid HealthCare Ltd., India. All reagents and solvents used were in analytical grade. For all the analysis, ultrapure water was purified using an Elix 3 coupled to a Milli-Q Gradient A10 system (Millipore, Bedford, USA). Purified water, 0.1 M HCl of pH 2.0, pH 3.0 glycine, pH 4.5 sodium acetate and pH 6.8 sodium phosphate buffer solutions were prepared according to USP Pharmacopoeia.[16]

Apparatus and conditions

The dissolution tests were performed in a Vankel (VK7025) auto sampler (VK8000) dissolution test system, multi-bath (n=6), in accordance with the United States Pharmacopeia (USP) general methods.[16] A Shimadzu Prominence HPLC system (Shimadzu, Kyoto, Japan) equipped with a SCL-10Avp system controller, LC-10 ADvp binary pump, DGU-14A degasser, CTO-10Avp column oven was used to keep the temperature at 40°C, SIL-10ADvp autosampler and a SPD-M10Avp UV detector was used. Detection was set at 210 nm and peak areas were integrated automatically by computer using a Shimadzu CLASS-VP chromatography software program. The stationary phase was a Prontosil KROMABOND, C18, 150 x 4.6 mm i.d., octadecyl silane chemically bonded to porous silica micro particles with a particle size of 5 µm and pore size of 120 Å. The Shimadzu Prominence HPLC system was operated isocratically at ambient temperature. The mobile phase was prepared by mixing buffer (2.73 gms of potassium dihydrogen orthophosphate was dissolved in 1000 mL milli-Q purified water and pH adjusted to 7.0±0.05 with potassium hydroxide solution) and acetonitrile in the ratio 45:55, v/v. The injection volume was 100 µl and the run time was 6 minutes. The mobile phase flow rate was 1.2 mL per minute. The mobile phase was filtered using a 0.45 µm membrane filter (Milipore, Milford, MA) and degassed with helium. Elico pH analyzer (Model: Elico LII20) was used to determine the pH of all solutions.

Dissolution test conditions

Solubility determination and sink conditions

Drug solubility is an important physical-chemical property to be considered when selecting the dissolution medium and can be determined by using an excess of the drug in the medium followed by stirring, filtration and quantification of the dissolved drug. Rasagiline mesylate sink conditions were applied and determined in different media: 0.1 M hydrochloric acid, pH 3.0 glycine buffer, pH

4.5 acetate buffer, pH 6.8 phosphate buffer and purified water. Vessels (n=3) containing 15.0 ml of medium were preheated to 37°C±0.5°C before adding an excess of rasagiline mesylate (10 mg). The solutions were maintained in the bath and stirred with a vortex at intervals of 10 minutes. An aliquot (5 mL) was removed from each vessel after 1 and 2 h and filtered through a Millipore® 0.45 µm membrane filter. One milliliter of the filtered aliquots were pipetted, diluted with medium at a final concentration 1.0 µg/mL and injected into the LC. Rasagiline mesylate standard solution having same concentration was prepared and injected. The proposed LC method with UV detection did not suffer interference by the formulation excipients and its degraded products, since no other peaks occurred in the same rasagiline mesylate retention time (Rt = 4.15 min), using the analytical conditions described above.

Stability determination

The solution stability was analyzed after 12 h at room temperature. Sample solutions were prepared in all different dissolution medium at the same conditions by the dissolution test. The proposed discriminative dissolution method was also applied to an accelerated stability study (40 ± 2°C/75 ± 5% RH) for 6 months. The method was applied to dissolution rate studies which were performed at 0, 3 and 6 months. A LC method with UV detection, developed in our laboratory was select to get a stability determination owing to its ability to separate rasagiline mesylate from the degraded products.

Filter evaluation

The filter evaluation is necessary to determine if it could be used in the dissolution test without adsorption of the drug and that it removes insoluble excipients that may otherwise cause high background or turbidity.[3] Standard and a sample solution were prepared in different dissolution media proposed with a final concentration of 1.0 µg/mL. The sample solutions were prepared using a placebo added an amount of reference standard equivalent to 1 mg of rasagiline mesylate in a beaker with 500 mL dissolution medium maintained at 37.0 ± 0.5 °C and stirred with a magnetic stirrer for 45 minutes. Aliquots of 10 mL were withdrawn at the same point and each one was centrifuged, filtered with a quantitative filter, a 0.45 µm nylon filter and a 3.0 µm filter. The standard solutions were prepared in volumetric flasks and the final solution was analyzed without filtration and filtered with the same filters listed above. All the filtrates were analyzed by LC method. For a filter to be acceptable for use, the results of the filtered portions are to approach (98-102%) the original concentrations of the unfiltered standard solution and the centrifuged sample solution.[3, 19]

Dissolution test and HPLC validation

All dissolution medium were used to be tested in the drug release percent in order to choose the most discriminatory one. The dissolution profiles were

obtained after the determination of the best dissolution condition tests. Dissolution testing was performed in compliance with USP[16] using paddles (apparatus 2); 500 mL of the different dissolution medium was maintained at 37 ± 0.5°C. Manual sampling aliquots of 10 mL were withdrawn at 5, 10, 15, 20, 30 and 45 minutes. The replacement of the same volume of the medium at 37±0.5°C was done for constant maintenance of the volume. Twelve samples were assayed for each dissolution profile. The withdrawn samples were filtered through 0.45 µm membrane filter and diluted the above test solution with concentration of 1.0 µg/ mL and injected to HPLC system for quantification at 210 nm. The standard solution, used in all dissolution tests, was prepared and obtaining the concentration of 1.0 µg/mL. The solution was filtered in 0.45 µm membrane filter before analysis.

After choice of best conditions, the validation of the LC method was performed. The LC method was selected because the excipients do not interfere with the maximum absorption of the drug and according to the literature,[3] it is the preferred method of analysis because it becomes an ideal and widely used method in characterization and quantitative analysis of drugs and their metabolites owing to its superior specificity, sensitivity and efficiency. Rasagiline mesylate standard chromatogram obtained in 0.1 M HCl demonstrated a maximum elution at 210 nm [Figure 2] and it was chosen as wavelength for dissolution test analysis.
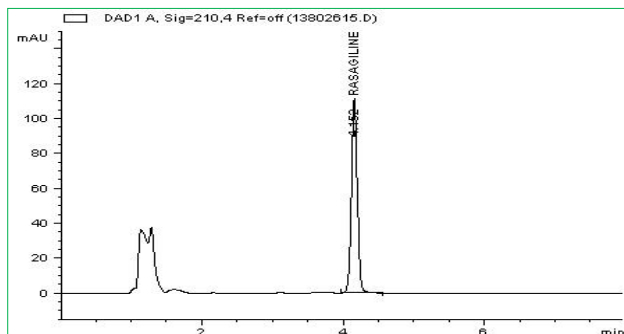

Figure 2: Chromatogram of rasagiline mesylate standard in 0.1 M HCl showing λ_{max} at 210 nm

The dissolution tests were validated to rasagiline mesylate tablets through the determination of specificity, linearity, precision, accuracy and robustness according to USP. [16]

Specificity

It was evaluated by preparing a placebo sample of commercial formulation of tablets in their usual concentration. The placebo sample was transferred to vessels with 500 mL of 0.1 M HCl deaerated and stirred at 37°C for 1 h at 150 rpm using paddle (USP apparatus 2). Aliquots of this solution were filtered with 0.45µ membrane filter and solutions were prepared using the same procedure described for sample solutions and injected three times.

Linearity

A stock solution of rasagiline mesylate (50 µg/mL) was prepared in 0.1 M HCl to give concentrations of 0.5, 0.75, 1.0, 1.25 and 1.5 µg/mL. Each solution was prepared in triplicate. The linearity was evaluated by linear regression analysis, which was calculated by the least square regression method and analysis of variance (ANOVA).

Precision

Repeatability and intermediate precision were used to assess the precision of the method. Repeatability was evaluated through relative standard deviation (% RSD) from the recovery data at 100% level.[3] The intermediate precision (inter-day) was evaluated by comparing the assays on two different days and using different analyst. The RSD was determinate. The recovery data were performed, in triplicate, using a well-characterized lot of the drug product of tight content uniformity. The dissolution test was done for 45 min using 500 mL of dissolution medium 0.1 M HCl, apparatus 2 rotating at 50 rpm.

Aliquots of 15 mL were filtered with 0.45µ membrane filter and analyzed by LC method at the same concentration (1.0 µg/mL), under the same conditions, during the same day and in two different days respectively. Each concentration was prepared in duplicate and each one was analyzed in triplicate.

Accuracy

A recovery study was evaluated by adding known amounts of rasagiline mesylate reference substance to placebo solution at 80, 100 and 120% of the nominal assay of Rasagiline mesylate. The dissolution test was done for 45 minutes using 500 mL of dissolution medium 0.1 M HCl, apparatus 2 rotating at 50 rpm. Aliquots of 15 mL were filtered with 0.45µ membrane filter and analyzed by LC spectrophotometric method at the final concentration 0.8, 1.0, and 1.2 µg/mL respectively. Each concentration was prepared in duplicate and each one was analyzed in triplicate.

Robustness

The robustness was tested by changing the following parameters of the HPLC method (one by one): mobile phase proportion – it was used pH 7.0 potassium dihydrogen orthophosphate buffer and acetonitrile (45:55, v/v) mobile phase; Change in pH of buffer – it was used pH 6.8 and pH 7.2; stationary phase – it was used a Develosil octa decyl silane UG (150 x 4.6 mm, 5 µm, Nomura Chemical Co Ltd., USA) and another HPLC system (Agilent 1200 series).

RESULTS AND DISCUSSION

The discriminatory power of the dissolution method depends on the method ability to detect changes in the drug product. Drug solubility and solution stability are important properties to be considered when selecting the dissolution medium.[3] The sink conditions tested shows

that rasagiline mesylate bulk is soluble in all the mediums tested, therefore the stable medium selected for dissolution test for rasagiline mesylate tablets is 0.1M HCl at the stirring speed of 50 rpm and the solubility of rasagiline mesylate in different media is shown in Table 1.

Table 1: Solubility of rasagiline mesylate in different media at 37±0.5°C

Medium	Solubility (1 h- µg/mL)	Solubility (2 h- µg/mL)
0.1 M Hydrochloric acid	437.5	474.2
Glycine buffer pH 3.0	429.2	436.1
Acetate buffer pH 4.5	448.5	425.2
Phosphate buffer pH 6.8	402.2	434.0
Purified water	353.8	381.7

The initial parameters for filtration and solution stability must be established prior to the completion of any dissolution samples.[20] The evaluation of the filters demonstrated that sample solutions filtered through 0.45 µm membrane filters are within 98-102% of the initial values and can be used in the dissolution tests in the different dissolution medium. To assess the stability of rasagiline mesylate in the dissolution medium, the samples are diluted in 0.1 M HCl, and tested after 24 h at room temperature. The solutions remain stable in all dissolution media tested for the time period specified and no degradation products are observed in any chromatogram. The tablets are also subjected to accelerated stability study (40 ± 2 °C) for 6 months and the results of stability of sample solutions are presented in Figure 3.

The sink condition tested shows that rasagiline mesylate bulk is soluble in, pH 2.0 HCl 0.1 M, glycine buffer pH 3.0, acetate buffer pH 4.5, phosphate buffer pH 6.8 and purified water. Then, dissolution test for rasagiline mesylate tablets are performed using these dissolution medium at the stirring speed of 50 rpm using paddle apparatus, to investigate the drug release in different dissolution medium. The dissolution profile obtained with different media tested was presented in Figure 4.
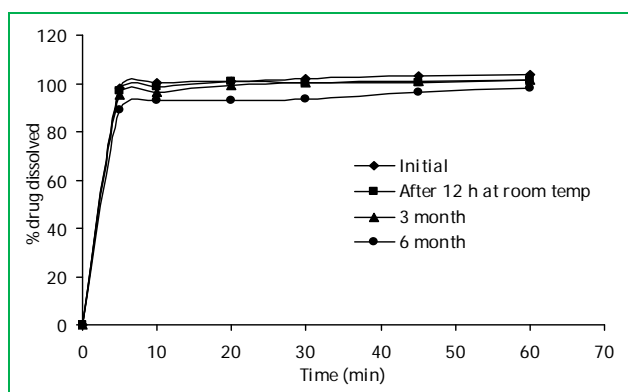

Figure 3: Dissolution profile of rasagiline mesylate tablets subjected to an accelerated stability study during 6 months, using 0.1 M HCl medium, and apparatus paddle at stirring rate of 50 rpm
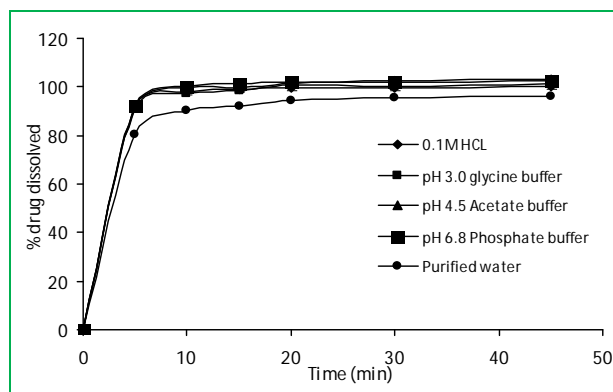

Figure 4: Dissolution profiles in different media (DPDM) of rasagiline mesylate using 500 mL medium with paddle apparatus at stirring rate of 50 rpm

Rasagiline mesylate shows high solubility and the drug product has demonstrated a rapidly dissolving in all the tested mediums. According to the literature [2, 3, 19] rapidly dissolving drug product is defined as one for which not less than 85% of the label claim is dissolved within 30 min, as tested using USP apparatus 2 at 50 rpm for 45 minutes and this product need not be subjected to a profile comparison if they can shown to release 85% or more of the active drug substance within 15 min.[16] For these types of products a one-point test will suffice. However the aim of our work is obtained the best conditions to development a discriminatory method, in this way when we used 0.1 M HCl as the dissolution medium.

The reversed-phase liquid chromatography method is developed and validated for dissolution of rasagiline mesylate tablets. The validation of analytical parameters described in guidelines[16] are evaluated. The type of method and its respective use shall be evaluated and it is also the responsibility of analyst to select parameters considered relevant for each method.

The specificity analysis revealed that the HPLC method do not suffer interference by the formulation excipients. The results obtained suggested that the HPLC method can be used for rasagiline mesylate tablets quantitation in dissolution tests, once the formulation excipients do not have any significant interference (does not exceed 2%) at 210 nm [Figure 5]. Thus, the HPLC method is useful to quantify rasagiline mesylate in pharmaceutical formulation from the dissolution tests.
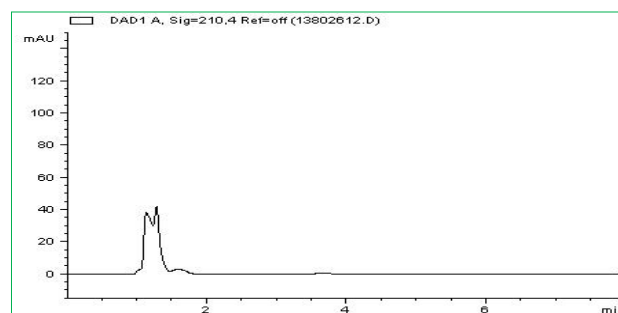

Figure 5: Chromatogram of placebo solution measured at 210 nm

To assess the linearity, three standard curves for rasagiline mesylate are constructed, plotting concentrations (µg/mL) versus absolute area (mV s) and shows good linearity on the 0.5-1.5 µg/mL range. The representative linear equation is y=11771x+268, where x is concentration and y is the peak absolute area. The correlation coefficient is r^2 = 0.999, indicating good linearity [Figure 6]. The data are validated by means of the analysis of variance, which demonstrated significant linear regression and no significant linearity deviation (p< 0.05).

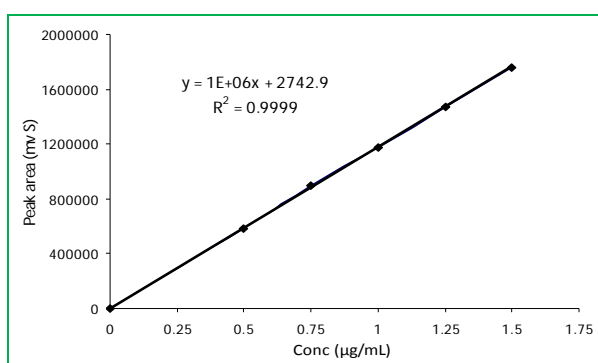**Figure 6:** Linearity/calibration curve of rasagiline mesylate

The precision of the dissolution tests is evaluated through repeatability and intermediate precision. The repeatability demonstrated % RSD for each day analyzed and the RSD for intermediate precision are in accordance with the data in the literature (0.9% in the first day and 0.2% in the second day after 45 min). According to Pharmacopoeial Forum[3] the RSD is above 20% in time points at 10 min or earlier, and at or above 10% RSD in later time points. These results can demonstrate the good precision of the method for dissolution test. These values are presented in Table 2.

The accuracy expresses the agreement between the accepted value and the value found. The accuracy is assessed from three replicate determinations of three different samples containing 0.8, 1.0 and 1.2 µg/mL of Rasagiline mesylate, giving concentrations respectively of 0.812, 1.056 and 1.230 µg/mL. The accuracy of the method is considered acceptable based on its intended use and % recovery is presented in Table 3.

The robustness of the method evaluated by changing the mobile phase (organic phase) proportion, pH of buffer solution (aqueous phase), the effect of change in column oven temperature and the last experiment is the quantitation in another liquid chromatograph (Agilent 1200 series, USA) where the retention time is a little high (about 4.28 minutes), but it is possible to quantify the drug satisfactorily, confirming the robustness of the method. At that rate, it is possible to demonstrate that the developed method is robust with all the changes employed. Standard and sample solutions are prepared as per the proposed method and injected into LC system. The system suitability parameters are given in Table 4.

Typical acceptance criteria for the amount of drug dissolved are in the range of 75% to 80% dissolved in 30 minutes. Acceptance criteria including test times are usually established on the basis of an evaluation of the dissolution profile data. [16] In this article, it is observed that for all products a dissolution of 90% is otained within 15 minutes. So, this acceptance criterion is utilized.

Table 2: Repeatability and intermediate precision of the dissolution method

Samples	% Rasagiline mesylate released/% RSD		
	Intra-day precision*		Inter-day precision*
	Day 1	Day 2	RSD (%)
5	91.8/12.3	89.3/10.4	0.8
10	97.6/5.4	95.6/3.2	0.6
15	99.3/2.1	98.9/1.2	1.0
20	99.6/0.7	100.2/1.1	1.1
30	99.7/0.4	100.6/0.9	0.5
45	100.3/0.9	101.2/0.2	0.2

*each value is the mean of 3 determinations

Table 3: Results from accuracy as recovery studies (*n* = 3) for rasagiline mesylate tablets

Sample	Amount of reference (µg/mL)		Recovery (%)	Mean (%)	[a]RSD (%)
	Added	Recovered			
R1	0.82	0.826	100.73	100.25	0.46
R2	1.03	1.028	99.80		
R3	1.22	1.223	100.24		

[a]relative standard deviation

Table 4: Robustness data of rasagiline mesylate tablets

System suitability parameters	Variation in organic phase ratio			Variation in pH of buffer solution			Variation in column oven temperature			Acceptance criteria
	90%	100%	110%	pH 6.8	pH 7.0	pH 7.2	35°C	40°C	45°C	
USP Tailing factor	1.0	1.1	1.1	1.1	1.1	1.2	1.1	1.1	1.2	NMT 2.0 %
USP Plate count	12134	2658	12022	11086	11240	10926	12214	12118	11562	NLT 2500
% RSD*	0.7	0.2	0.4	0.2	0.8	0.5	0.3	0.7	1.1	NMT 2.0 %

*each value is the mean of replicate (n=5) determinations

CONCLUSION

The appropriate dissolution test developed and validated for rasagiline mesylate tablets are considered satisfactory. It is carefully studied in order to guarantee the drug stability during all the analysis time. This study concludes that to use the most discriminating conditions for dissolution testing of rasagiline mesylate tablets, a 0.1 M HCl, paddles, stirring speed of 50 rpm and 45 minutes of test time provides satisfactory results for all products appear to be the best condition contributing to assure the therapeutic efficacy of the drug. The liquid chromatographic method is the advanced analytical method for dissolution test because the analyses are sensitive to determine the drug in lower concentration; the results can be obtained faster. It was validated and showed to be specific, linear, precise, accurate and robust. The method demonstrated to be adequate to be used in quality control of rasagiline mesylate tablets since there is no dissolution test indicated in official monograph, collaborating to the official codes.

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