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Development and validation of a reverse phase liquid chromatography method for the quantification of rasagiline mesylate in biodegradable PLGA microspheres

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ABSTRACT

In the present study, a reverse phase high performance liquid chromatographic method was developed and validated for the determination of rasagiline mesylate in biodegradable microspheres. Chromatographic separation was carried out on a RP-18 column using a mobile phase consisting of acetonitrile:water (5:95, v/v) adjusted at pH 3.1. Flow rate was 1.0 ml min^{-1} and UV detection at 290 nm. Acyclovir was used as the internal standard. The calibration curve was linear over the range 0.5–20.0 μg ml^{-1}. R.S.D. for precision was <1.8%. Accuracy ranged between 99.01% and 102.55% with a R.S.D. lower than 1.3%. LOD and LOQ were 0.07 μg ml^{-1} and 0.23 μg ml^{-1}, respectively. The method was simple, rapid, and easy to apply, making it very suitable for routine analysis of rasagiline mesylate in biodegradable PLGA microspheres. It could be also used with reliability for the determination of the drug in other pharmaceutical dosage forms.

1. Introduction

Parkinson's disease (PD) is a degenerative, late-onset human neuropathology often marked by hypokinesia, resting tremor, muscle rigidity and general muscle weakness. PD affects one or two adults in every thousand, and the risk increases beyond 50 years old. The main neuropathological hallmark of PD is a degeneration of nigro-striatal dopaminergic neurons. Inhibitors of monoamine oxidase (MAO) with selectivity and specificity for MAO type B (MAO-B) prolong the activity of both endogenously and exogenously derived dopamine, making them an option either as monotherapy in early PD or as adjunctive therapy in patients treated with levodopa that are experiencing motor complications. In addition to symptomatic benefits, experimental data suggest that MAO-B inhibitors may be neuroprotective through MAO-B inhibition and other mechanisms that have yet to be clearly defined [1,2].

The two available MAO-B inhibitors approved for use in the United States, rasagiline and selegiline, each provide symptomatic relief as monotherapy and as adjunctive therapy, and have shown potential disease-modifying effects in experimental models and clinical studies. Selegiline has a limited potency and it produces amphetamine metabolites that result in adverse effects and interfere with any putative disease-modifying effects. Rasagiline (Fig. 1) is more potent than selegiline, exhibits disease-modifying effects in experimental models, lacks amphetamine metabolites and also exhibits neuroprotective activity [3–10].

Several studies have documented the efficacy and tolerability of rasagiline as monotherapy of early stage disease [11] and as adjunctive therapy with L-dopa in moderate to advances disease [12,13].

Rasagiline is rapidly absorbed by the gastrointestinal tract, reaching the peak plasma concentration in 30 min. However, the oral bioavailability is only 36%, with a very short elimination half-life (0.6–2 h) [14]. These characteristics, the fact that rasagiline is used in a chronic treatment and that at the present moment it does not exist a controlled release system make rasagiline a suitable candidate for the development of these therapeutic systems.

Microspheres implanted directly into the brain tissue allow the possibility of high levels of drug concentrations confined to the region of interest [15,16], with reduced systemic toxicity when compared to intravenous administration [17]. Moreover, microspheres elaborated with biodegradable and biocompatible polymers, such as poly-D,L-lactic-co-glycolic acid (PLGA), have the advantage of disappearing from the region of interest once they have exerted their therapeutic effect.

For these reasons it is essential to analyze and quantify the drug (rasagiline) in the polymeric system (microspheres) which can be considered as a major part of the quality control and the standardization of the controlled release system developed.

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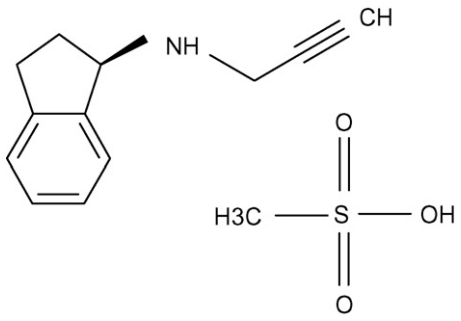

Fig. 1. Chemical structure of rasagiline mesylate.

In the literature there are described only two analytical methods for the determination of rasagiline in biological fluids. One of these methods is a gas chromatography–mass spectrometry procedure in electron impact mode [18]. This method, sparsely described in a pharmacokinetic–pharmacodynamic study, is used for the quantification of the drug in biological samples and presents a limit of quantification of 0.25 ng/ml and, accuracy and precision with coefficients of variation and percentage of error lower than 20%. The other method uses a liquid chromatograph equipped with an amperometric electrochemical detector with dual glass carbon electrodes [19], with no information regarding its analytical performance given. These procedures therefore require detection devices not generally available in routine pharmaceutical analysis laboratories. In this work, we have therefore developed and validated a new analytical method for the determination of rasagiline mesylate, which can be applied for the determination of the drug in biodegradable microspheres. The HPLC method described is simple, rapid, sensitive, and precise and utilizes acyclovir as an internal standard. UV detection is performed at 290 nm.

2. Experimental

2.1. Reagents and chemicals

Rasagiline mesylate was obtained from Hangzhou Onion Chemical Co. Limited (China). Acyclovir (used as internal standard (IS)) was purchased from Guinama (Spain). Poly-D,L-lactic-co-glycolic acid (Resomer-502®) was obtained from Boehringer Ingelheim Chemicals Division (Germany). Polyvinyl alcohol (PVA) 72,000 Da was purchased from Merck (Germany). Water was purified by Milli-Q filtration system (Millipore, USA). All reagents and solvents used were of analytical and purchased from Panreac (Barcelona, Spain).

2.2. Preparation of rasagiline mesylate-loaded microspheres

Microspheres were prepared by a water–oil–water (W/O/W) multiple emulsion solvent evaporation. Briefly, an aqueous solution of rasagiline mesylate (15 mg dissolved in 150 μl buffer phosphate solution, pH 3.3) was emulsified into a solution of polymer (450 mg) in methylene chloride (1.5 ml) by sonication for 30 s under ice-cooling (sonicator, Heat Systems, Ultrasonics Inc., Plainview, NY). The resulting water–oil (W/O) emulsion was emulsified into the external phase (800 ml PVP solution, 0.25% w/v, with NaCl 0.5N, and alkalinized with NaOH 1N to pH 10) and stirred using a polytron (Kinematica GmbH PT 300, Germany) for 20 s at 6500 rpm to form a W/O/W emulsion. The double-emulsion was stirred with a magnetic stirred for 3.5 h at room temperature. The solid microspheres were vacuum-filtered through a 5 μm membrane filter (Sartorius, Germany), washed three times with water and vacuum-dried for at least 12 h. PLGA microspheres (blank microspheres) were also prepared.

2.3. Instrumentation

A liquid chromatograph (HPLC) Waters (USA) with a pump model 510 Waters (USA), a UV detector model 490E Waters (USA), an autosampler 712D WTSP Waters (USA), an Empower Login HPLC System Manager Software Waters (USA) was used. A C18 column Mediterranean Sea (particle size 5 μm, 250 mm × 4 mm) (Teknokroma S. Coop., Barcelona, Spain) was used.

2.4. Chromatographic conditions

The composition of the mobile phase was acetonitrile:water (5:95, v/v) (adjusted to pH 3.1 with orthophosphoric acid). The mobile phase was vacuum-filtered through 0.45 μm nylon Millipore membranes (Millipore, USA), and degassed by ultrasonication for 20 min before use. The mobile phase flow was set at 1.0 ml min^{-1} and the injection volume was 20 μl. After equilibration with the solvent to obtain a stable baseline, aliquots of samples were injected. The total run time was 15 min. The absorbance of the eluent was monitored at 290 nm with a detection sensitivity of 0.250 aufs. Acyclovir (30.1 μg ml^{-1}) was used as internal standard. All the analyses were performed at 25.0 ± 0.5 °C.

2.5. Standards and sample solutions preparation

Standard stock solutions of rasagiline mesylate (1000 μg ml^{-1}) and acyclovir (IS) (700 μg ml^{-1}) were prepared in water. These solutions were kept stored under refrigeration (4.0 ± 0.5 °C). Working standard solutions were freshly prepared daily by appropriate dilution of the stock solutions with mobile phase.

Sample solutions were prepared by weighing a fixed amount of rasagiline-loaded PLGA microspheres (30 mg) which was dissolved in 5 ml of methylene chloride. Acyclovir was used as internal standard at a concentration of 0.12 mg ml^{-1}. Rasagiline mesylate and the internal standard were extracted from methylene chloride three times with 5 mL PBS (pH 7.4). Previously, different volumes of PBS were tested to optimize the extraction procedure. The mixture was centrifuged (Universal 32, Hettich, Germany) at 6000 × *g* for 5 min and the supernatant was extracted. The supernatant was then transferred to a 20 ml volumetric flask and diluted with mobile phase to complete the volume. All samples were filtered through 0.45 μm filters before HPLC analysis.

2.6. Assay validation

Method validation was carried out under the International Conference on Harmonization (ICH) guidelines for validation of analytical procedures [20]. The assay was validated with respect to linearity, precision, accuracy, selectivity, sensitivity and robustness. The stability of rasagiline mesylate was also studied. For this, analysis of variance (ANOVA) was used to verify the validity of the method.

2.6.1. Linearity

Calibration curves were obtained from five different drug concentrations (0.5, 1.0, 5.0, 10.0 and 20.0 μg ml^{-1} of rasagiline mesylate). The solutions were made in triplicate and each solution was injected three times. The curves were generated by plotting the peak area ratios between rasagiline mesylate and internal standard against rasagiline mesylate concentration. Linearity was evaluated by linear regression using ANOVA.

2.6.2. Precision

The precision of the method was determined by instrumental precision, repeatability (intra-day) and intermediate precision (inter-day) and was expressed as relative standard deviation

(R.S.D.). Instrumental precision was determined by performing six replicate injections of a sample of 10.0 μg ml^{-1} of rasagiline mesylate. Repeatability was evaluated by analysis of three concentration levels (0.5, 10.0 and 20.0 μg ml^{-1} rasagiline mesylate) the same day. Each solution were made in triplicate and injected three times the same day under the same experimental conditions. The intermediate precision (inter-day precision) was determined by analyzing three independent samples in triplicate during three different days.

2.6.3. Accuracy/recovery

The accuracy of an analytical method is the closeness of test results obtained by that method to the true value. In this study, accuracy was determined based on the recovery (percentage) of known amounts of rasagiline mesylate. This was performed by analyzing rasagiline mesylate at three concentration levels (0.5, 10.0 and 20.0 μg ml^{-1}), all with a constant concentration of 30.1 μg ml^{-1} of internal standard. Samples were prepared in triplicate. The accuracy of the assay was determined by comparing the found concentration with the added concentration, taking into consideration that rasagiline mesylate purity was 99.2% as indicated by the manufacturer.

2.6.4. Selectivity

Method selectivity was evaluated through possible interference due to components of the biodegradable microspheres. For this, three different batches of blank PLGA microspheres were prepared and then analyzed by the HPLC procedure with a run time of 30 min.

In addition, the selectivity of the method was verified through degradation studies, where the standard solutions (10.0 μg ml^{-1} of rasagiline mesylate and 30.1 μg ml^{-1} of internal standard) were subjected to different degrees of stress as shown in Table 1.

2.6.5. Sensitivity

Sensitivity of the method was determined by means of the detection limit (LOD) and quantification limit (LOQ). The LOD and LOQ were measured based on the method described by the International Conference on Harmonization [20]. Calculations for LOD were based on the standard deviation of the calibration curve (σ) and the slope of curve (S), using the equation LOD = $3.3 \times \sigma/S$. LOQ was calculated by the equation LOQ = $10 \times \sigma/S$. Both LOD and LOQ were calculated using three concentrations of rasagiline mesylate (0.3, 0.4 and 0.5 μg ml^{-1}).

2.6.6. Robustness

Robustness of the method was evaluated by the analysis of several batches of microspheres under different experimental conditions such as changes in the composition of the mobile phase, column temperature, flow rate and its pH. The percentage of acetonitrile in the mobile phase was varied ±1%, the column temperature was varied ±5 °C, the flow rate was varied ±0.2 ml min^{-1} and, pH of the mobile phase was changed ±0.2 units. Besides, the method was applied using different analytical columns C18 (Tracer, Lichrospher and Nucleosil) (Teknokroma S. Coop., Barcelona, Spain). Their effects on the retention time (t_R), tailing factor (T), resolution of the peaks (R), recovery and repeatability were studied.

2.6.7. Stability

The stability of rasagiline mesylate in solution was studied after storage at 40 °C for 15 days. Six solutions containing 20.0 μg ml^{-1} of rasagiline mesylate were tested. Quantification was performed after preparation and at 7 and 15 days thereafter.

In addition, the stability of rasagiline mesylate was studied in samples of PLGA-loaded microspheres after sterilization. For this, samples were irradiated by using Co-60 as irradiation source (Aragogamma S.A., Barcelona, Spain) and received a dose of 25 kGy, considered as adequate for the purpose of sterilising pharmaceutical products when the bioburden is not known, according to the European Pharmacopoeia.

3. Results and discussion

3.1. Optimization of the chromatographic method

The chromatographic conditions were adjusted to provide the best performance of the assay. For system optimization the main important parameters such as type and concentration of organic modifier, pH, IS and, mobile phase flow rate were investigated.

3.1.1. Effect of pH

Different pH values of the mobile phase were checked to establish the optimum separation and highest analytical sensitivity for rasagiline mesylate and the IS. The mobile phase was buffered because of the existence of strongly ionizable groups in the chemical structure of the drug which could ionize at acidic and alkaline pH values. The pH values tested were 2.6, 3.1, 3.6, 6.0, 7.9 and, 8.5. Finally, the best results were obtained at pH 3.1 ± 0.2 by using orthophosphoric acid. The choice of this pH for the mobile phase is justified by the excellent symmetry of the peaks and the adequate retention times of rasagiline mesylate and the IS.

3.1.2. Effect of mobile phase composition

To reduce the complexity of analytical conditions for continuous measurements we tested two different organic solvents (acetonitrile and methanol). For this, both water–acetonitrile and water–methanol mixtures were tested as mobile phases. It was observed that the water–acetonitrile system gave a better resolution and peak symmetry than the water–methanol system, providing suitable retention times for rasagiline mesylate and IS.

To choose the mobile phase, different proportions of water–acetonitrile (95:5, 90:10, 80:20, 70:30, 60:40, 50:50, 40:60, 30:70, v/v) were tested and evaluated before the final chromatographic conditions were selected. As a result, modification of rasagiline mesylate retention times occurred from 3.8 min to 1.15 min as the percentage of acetonitrile increased. Finally, we have chosen as mobile phase water–acetonitrile (95:5, v/v) (adjusted to pH 3.1 ± 0.2 with orthophosphoric acid). As a result, the standard solutions of rasagiline mesylate and IS showed symmetric and well-defined peaks, with an average retention time for rasagiline mesylate of 3.9 min and 6.3 min for the IS (Fig. 2). Resolution between peaks was 4. Tailing factor was 1.20 for rasagiline mesylate and 1.23 for IS.

Table 1
Results of the stress conditions experiments.

Stress condition	Stress treatment	t_R degradation products (min)	R
Acid	0.1N HCl, 60 °C, 24 h	4.312	1.8
Basic	0.1N NaOH, 150 °C, 30 min	8.336	2.1
Oxidative	3% H_2O_2, room temperature, 24 h	12.577	8.2
Sunlight degradation	Sunlight, room temperature, 12 h	5.150	2.1

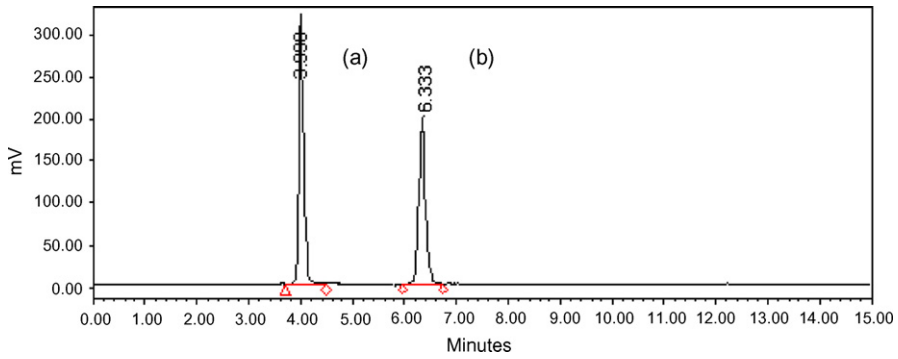

Fig. 2. Typical chromatogram corresponding to a standard solution of rasagiline mesylate/internal standard: (a) rasagiline mesylate (10.0 μg ml^{-1}) and (b) internal standard (30.1 μg ml^{-1}).

Table 2
System suitability results of the proposed method.

Analyte	*R*	*N*	*T*	R.S.D.	
				t_R	Peak area
Rasagiline mesylate	10.5	6434	1.20	0.31	0.37
IS	10.5	8700	1.10	0.37	0.97
Required limits	$R > 2$	$N > 2000$	$T < 1.5$	R.S.D. < 1%	

3.1.3. Effect of flow rate

Different mobile phase flow rates (0.8, 1.0 and 1.2 ml min^{-1}) were investigated. The optimum flow rate for which the column plate number was maximum, with the best resolution between all components and with a short run time (<10 min) was 1.0 ml min^{-1}.

3.1.4. Internal standard

Different compounds were tested as internal standard (IS) for the chromatographic procedure. Among them, benzoic acid, sodium benzoate, DL-tyrosine, ascorbic acid, lidocaine, caffeine, pseudoephedrine sulphate and phenylephrine hydrochloride were tested. Benzoic acid, sodium benzoate, lidocaine, caffeine, pseudoephedrine sulphate, DL-tyrosine and, phenylephrine hydrochloride did not elute before 20 min with the selected mobile phase, thereby prolonging run time. Ascorbic acid and acyclovir eluted before 10 min of the analysis however, of these two compounds acyclovir is the one that presents a better symmetry and resolution with respect to rasagiline mesylate. Therefore, acyclovir has been chosen as IS.

3.2. Method validation

3.2.1. System suitability

System suitability was performed to confirm that the equipment was adequate for the analysis to be performed. The test was carried out by making six replicate injections of a standard solution containing 10.0 μg ml^{-1} and 30.1 μg ml^{-1} of rasagiline mesylate and IS, respectively, and analyzing each solute for their peak area, theoretical plates (*N*), resolution and, tailing factor. The results of system suitability in comparison with the required limits are shown in Table 2. The proposed method fulfils these requirements within the accepted limits.

3.2.2. Linearity

The standard calibration curve was linear over the concentration range 0.5–20.0 μg ml^{-1}. The correlation coefficient obtained after linear regression analysis was 0.9999 (Table 3). The equation of the calibration curve based on the peak ratio of rasagiline mesylate/internal standard with respect to rasagiline mesylate concentration was $y = 0.11635x - 0.0020$. The standard error was 0.0080. According to statistical analysis by ANOVA, the curve was linear with $p < 0.005$.

3.2.3. Precision

Precision tests performed under the same experimental conditions (instrumental, intra-day and inter-day) were expressed by the corresponding R.S.D. values. These values are shown in Table 4. For instrumental precision the R.S.D. value was 1.15% which was considered acceptable. The R.S.D. of repeatability (intra-day) and intermediate precision (inter-day) ranged between 0.17% and 1.78%.

Table 3
Statistical analysis of linearity.

Multiple regression analysis						
Parameter	Estimation	Standard error	*t*-Statistic	*p*-Value	Lower 95%	Upper 95%
Intercept	−0.0020	0.0029	−0.6852	0.5252	−0.0083	0.0043
Slope	0.1164	0.0003	407.871	0.0000	0.1157	0.1170

ANOVA					
Parameter	Sum of squares	Degrees of freedom	Mean of squares	*F*	*p*-Value
Regression	10.5505	1	10.5505	166314.66	0.0000
Residual	0.0008	13	0.0001		
Total	10.5513	14			

Correlation coefficient	0.9999
R^2	0.9922
Adjusted R^2	0.9916
Standard error	0.0080

Table 4
Summary of precision determined during method validation.

Concentration ($\mu g\,ml^{-1}$)	R.S.D. (%), intra-day			R.S.D. (%)
	Day 1[a]	Day 2[a]	Day 3[a]	Inter-day[b]
0.5	0.74	1.78	1.31	1.32
10.0	0.35	0.96	0.68	0.93
20.0	0.52	0.17	0.56	0.50

[a] Analyzed on the same day ($n = 3$).
[b] Analyzed on three consecutive days ($n = 9$).

Table 5
Accuracy of the method determined according to ICH Q2.

Concentration ($\mu g\,ml^{-1}$)		Recovery (%)[a]	R.S.D. (%) ($n = 3$)
Added	Recovered		
0.500	0.513	102.55	
0.500	0.501	100.18	
0.500	0.512	102.37	1.30
10.000	9.991	99.91	
10.000	10.082	100.82	
10.000	9.901	99.01	0.91
20.000	19.999	99.99	
20.000	19.868	99.34	
20.000	20.128	100.64	0.65

[a] (Found concentration/added concentration) × 100.

These values show a low variability between the values obtained for each concentration.

3.2.4. Accuracy

The results of the accuracy studies are shown in Table 5. Recovery ranged between 99.01% and 102.55% with R.S.D. less than 1.3%. The values obtained show a suitable accuracy for the analytical method.

3.2.5. Selectivity

Selectivity was checked with respect to interferences among components of the polymeric system (blank PLGA microspheres). Three different samples of blank PLGA microspheres were analyzed according to the proposed method and checked for any interference at the retention times of rasagiline mesylate and IS. The chromatogram of blank PLGA microspheres showed no peaks in the region in which rasagiline mesylate and IS eluted, indicating specificity of the method against polymer interference. The chromatograms obtained for rasagiline mesylate-loaded PLGA microspheres showed well-resolved and symmetric peaks corresponding to the drug and IS (Fig. 3) being both compounds eluted at the same retention times obtained for the solution containing rasagiline mesylate and IS (Fig. 2).

Degradation products were not detected when samples were exposed to acid and basic degradation at room temperature (data not shown). Therefore, samples were exposed to additional stress conditions sufficient to degrade the analyte. Table 1 shows the degradation products obtained when samples were subjected to these additional degrees of stress. Under these stress conditions, degradation products were formed however, they were well resolved ($R > 1.8$) from rasagiline mesylate and IS. An example of specificity criteria for an assay method is that the analyte peak will have baseline chromatographic resolution of at least 1.5 from all other sample components, taking into consideration that the peaks can be integrated separately as in our case (acid stress conditions) [21]. As examples of the stress experiments, Figs. 3 and 4 show the chromatograms corresponding to strong acidic and oxidative conditions tested, respectively. The samples showed sufficient degradation in 0.1N HCL at 60 °C for 24 h with the major degradation product obtained at 4.312 min (Table 1). When samples were exposed to 0.1N NaOH at 150 °C for 30 min the major product appeared at 8.33 min (Table 1). Under oxidative stress conditions (3% H_2O_2, room temperature and 24 h) one peak was obtained at 2.44 min which corresponds to H_2O_2 and with the major degradation product appearing at 12.57 min (Fig. 5). Finally, exposure of samples to sunlight at room temperature for 12 h resulted in a degradation peak obtained at 5.15 min (Table 1).

Therefore, our results confirm the selectivity of the proposed method.

3.2.6. Sensitivity

LOD and LOQ were 0.07 $\mu g\,ml^{-1}$ and 0.23 $\mu g\,ml^{-1}$, respectively. These values are adequate for the detection and quantification of rasagiline mesylate.

3.2.7. Robustness

Quality control of several batches of microspheres was used to evaluate robustness of the method as shown in Table 6. During these assays, the retention times were not significantly changed and peak symmetry was maintained. These facts suggest that the method did not change with time and experimental conditions. However, it could be noted that organic composition of the mobile phase and flow rate are the factors that had more influence on method performance.

3.2.8. Stability

Chromatograms obtained for solutions containing 20 $\mu g\,ml^{-1}$ of rasagiline mesylate after storage for 15 days at 40 °C did not show any other peaks corresponding to degradation products after elution for 30 min. Moreover, peak areas and retention times for rasagiline mesylate were not significantly changed in this

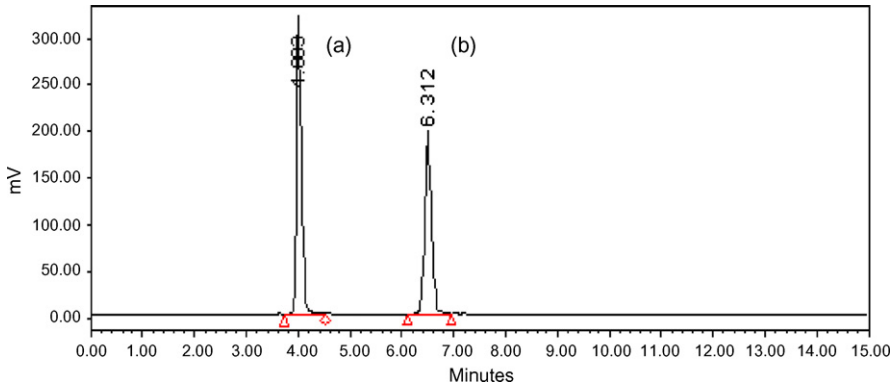

Fig. 3. Typical chromatogram corresponding to blank PLGA microspheres loaded with rasagiline mesylate and internal standard: (a) rasagiline mesylate (10.0 $\mu g\,ml^{-1}$) and (b) internal standard (30.1 $\mu g\,ml^{-1}$).

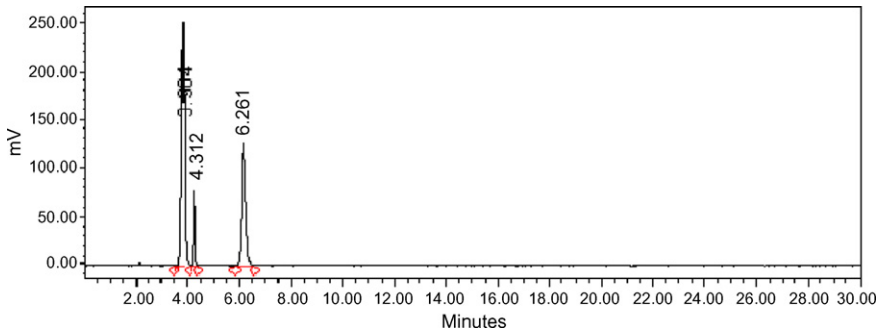

Fig. 4. Typical chromatogram corresponding to results obtained under acid stress conditions.

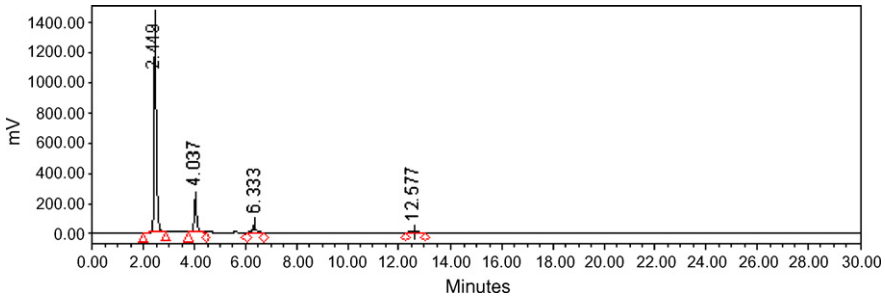

Fig. 5. Typical chromatogram corresponding to results obtained under oxidative stress conditions.

Table 6
Robustness of the method.

Parameter	Value	*R*	*T*	t_R (min)	Recovery (%)	R.S.D. (%)
pH	2.9	10.1	1.17	4.074	97.6	0.75
	3.1	10.5	1.20	4.029	100.0	0.36
	3.3	11.3	1.25	4.204	101.1	0.21
Flow rate (ml min^{-1})	0.8	11.6	1.35	5.020	98.2	0.23
	1.0	10.5	1.20	4.029	100.0	0.36
	1.2	9.8	1.19	3.381	98.4	0.31
Mobile phase (% acetonitrile)	4	14.3	1.28	4.668	98.7	0.24
	5	10.5	1.20	4.029	100.0	0.36
	6	17.2	1.13	5.527	98.5	0.16
Column temperature (±0.5 °C)	25	10.5	1.20	4.029	100.0	0.36
	30	11.3	1.18	3.929	99.6	0.26
	35	10.8	1.13	3.851	99.2	0.08

stability study. Moreover, when samples of sterilized rasagiline mesylate-loaded PLGA-microspheres were analyzed by HPLC, no other interfering peaks appeared in the chromatograms.

3.2.9. Application of the method

This method was used for the quantification of rasagiline mesylate from PLGA biodegradable microspheres. Sextuplicate measurements were performed for each sample. Encapsulation efficiency (EE) was calculated as the ratio of the actual drug content in the microspheres over the theoretical drug loading:

$$\text{EE}(\%) = \frac{\text{Drug content in the microspheres}}{\text{Theoretical drug loading}} \times 100$$

After analysis of seven samples, encapsulation efficiency of rasagiline mesylate within PLGA microspheres was found to be 97.95 ± 2.58% (Table 7).

Table 7
Encapsulation efficiency.

#	EE (%)
1	98.80
2	95.53
3	99.58
4	92.93
5	101.26
6	98.26
7	100.80
Mean	97.95
R.S.D.	2.58

4. Conclusions

This is the first developed and validated reverse phase liquid chromatography method for the determination of rasagiline mesylate using UV detection. The method was validated in terms of linearity, precision, accuracy, selectivity, detection limit and quantification limit and robustness and can be used for the quantification of rasagiline mesylate from PLGA biodegradable microspheres. The present HPLC method can be considered simple, rapid, and easy to apply, making it very suitable for routine analysis of rasagiline mesylate in biodegradable PLGA microspheres. It involves a single-step procedure for the preparation of the samples and direct

injection. Sample preparation and analytical procedure run times are short (less than 15 min) and moreover, a low percent of organic solvent (acetonitrile 5%) is used in the composition of the mobile phase. This method has been developed for detection and quantification of rasagiline mesylate in microspheres however; it could be also used with reliability for determination of the drug in other pharmaceutical dosage forms. Therefore, this method can be proposed for routine analysis in quality control.

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References

[1] P.A. LeWitt, D.C. Taylor, Neurotherapy 5 (2008) 210–225.
[2] H.H. Fernández, J.J. Chen, Pharmacotherapy 27 (2007) 1745–1855.
[3] D. Guay, Am. J. Geriat. Pharmacother. 4 (2006) 330–346.
[4] J.J. Chen, Formulary 41 (2006) 18–24.
[5] J.J. Chen, J. Clin. Pharmacol. 45 (2005) 878–894.
[6] O. Bar-Am, M. Yogev-Falach, T. Amit, Y. Sagi, M. Youdim, J. Neurochem. 89 (2004) 1119–1125.
[7] O. Bar Am, T. Amit, M. Youdim, Neurosci. Lett. 355 (2004) 169–172.
[8] N. Stefanova, W. Poewe, G. Wenning, Exp. Neurol. 210 (2008) 421–427.
[9] M. Stern, K. Marek, J. Friedman, R. Hauser, P. LeWitt, D. Tarsy, W. Olanow, Mov. Disord. 19 (2004) 916–923.
[10] M. Youdim, A. Gross, J. Finberg, Br. J. Pharmacol. 132 (2001) 500–506.
[11] Parkinson Study Group, Arch. Neurol. 59 (2002) 1937–1943.
[12] Parkinson Study Group, Arch. Neurol. 62 (2005) 241–248.
[13] Parkinson Study Group, Lancet 365 (2005) 947–954.
[14] J. Chen, D. Swope, K. Dashtipour, Clin. Ther. 29 (2007) 1825–1849.
[15] R. Langer, J. Control. Release 16 (1991) 53–59.
[16] B. ArVca, H.S. KaY, A. Moghdam, N. Akalan, A.A. HVncal, Eur. J. Pharm. Sci. 8 (1999) 5–6.
[17] R.J. Tamargo, H. Brem, Neurosurgery 2 (1992) 259–279.
[18] J. Thébault, M. Guillaume, R. Levy, Pharmacotherapy 24 (2004) 1295–1305.
[19] J. Finberg, I. Lamensdorf, J. Commissiong, M. Youdim, J. Neural Transm. 48 (1996) 95–101.
[20] International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH), Q2B: Guideline on Validation of Analytical Procedure-Methodology, London, November 1996.
[21] G.A. Shabir, J. Chromatogr. A 987 (2003) 57–66.