Review Article

REVIEW OF RASAGILINE ESTIMATION BY DIFFERENT ANALYTICAL METHODS AND BIOLOGICAL MATRICES

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ABSTRACT

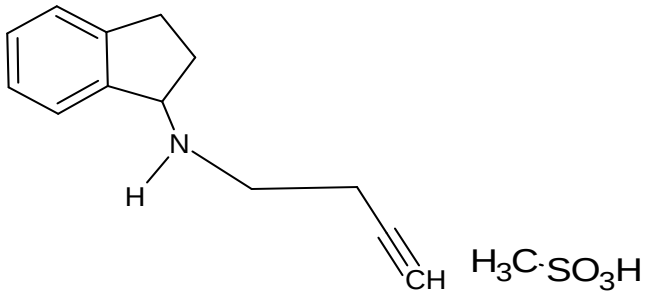
Rasagiline is a Monoamine oxidase-B (MOA-B) inhibitor that binds covalently to the N5 nitrogen of the flavin residue of MAO, resulting in irreversible inactivation of the enzyme. Use for the treatment of Parkinson's disease, which is a progressive, degenerative neurological disorder characterized pathologically by the selective loss of dopaminergic neurons in the substantia nigra pars compacta. This review article discusses the estimation of Rasagilline by different analytical methods in pharmaceutical preparations and biological matrices. Several spectrophotometric UV-Vissible, High-performance thin layer chromatography, and capillary electrophoresis analysis methods are used to determine Rasagilline in raw materials. And also, high-performance liquid chromatography (HPLC) by gradient and isocratic elution techniques, Ultra high pressure liquid chromatography (UHPLC) and difference between HPLC and UHPLC methods, and hyphenated techniques LC-MS methods can be used to analyze Rasagilline in biological matrices (plasma).

Keywords: Rasagilline, Parkinson's disease, spectrometry, HPLC, UHPLC, LC-MS, Electrophoresis.

INTRODUCTION

Rasagiline is second generation selective irreversible MOA-B inhibitor that binds covalently to the N5 nitrogen of the flavin residue of MAO and resulting in irreversible inactivation of the enzyme used in the treatment of Parkinson's disease[1]. Parkinson's disease is a progressive, degenerative neurological disorder characterized pathologically by the selective loss of dopaminergic neurons in the substantia nigra pars compacta and intracellular accumulation of Lewy bodies. The loss of dopamine neurons leads to the characteristics of motor symptoms [eg: bradykinesia, hypokinesia, muscle rigidity, postal instability] of Parkinson's disease and also experience non-motor symptoms [eg:autonomic dysfunction, orthostatic hypotension, depression anxiety and sleep distributable]. Current therapies against PD are pharmacological therapies levodopa treatment. L-DOPA treatment, which focuses on alleviating symptoms, restoring dopamine levels, modifying motor symptoms[2]. Parkinson's disease (PD) is one of the most common neurodegenerative disorders and is the second after Alzheimer's disease. This disease affects mostly older people, but early onset of Parkinson's disease is observed in people aged 40-50 years[3].

Rasagiline is chemically known as (R)-N-(Prop-2-ynyl)-2,3dihydro 1-H-inden-1-amine



Molecular formula (C12H13N) CH4SO3., Molecular weight 267.34 g/mol, Characteristics White to off-white, crystalline solid Rasagiline is freely soluble in water, ethanol and sparingly soluble in isopropyl alcohol. It is a chiral compound with one asymmetric carbon atom in a five-member ring with an absolute R-configuration which is produced as a single enantiomer.

UV-VISIBLE SPECTROSCOPY

UV spectroscopy

The quantitative determination of rasagiline mesylate in bulk and dosage form was carried out by simple, rapid, and precise UV spectroscopy method with the beers range of 50-300µg/ml, and the absorbance maxima was found at 263nm in 0.1M sulphuric acid, and this method was validated statistically. The %R.S.D was found to be less than 2%, Recovery was found to in the range of 98-102%. By this validation, the above method was economical and sensitive for estimation of Rasagiline mesylate in bulk and tablet form[4]

Visible spectroscopy

The UV-Vis spectroscopic method shows simple, rapid, and accurate determination of Rasagiline in pharmaceutical preparations by various. In the frist method, the determination depends on the reaction of Rasagilline with chloranilic acid. The colored solution was quantitated at 524 and shows the Linearity range with (0.9988–0.9996) and good correlation coefficients were observed as 25–300 μgmL−1. In the second method, the determination depends on the reaction of Rasagilline with tetrachloro-1,4-benzoquinone. The colored solution was quantitated at 535 and Linearity ranges for the above solution with good correlation coefficients (0.9988–0.9996) were observed as 25–350μgmL−1. In third method the determination depends on the reaction of Rasagilline with 7,7,8,8- tetra cyanoquinodimethane and The colored solution were quantitated at 843 nm and Linearity ranges for a solution with good correlation coefficients (0.9988–0.9996) were observed as 50–500μgmL−1.

Table 1

Sl. no	Coloured product	Wave length	Concentration	Linearity range
1	Rasagilline with chloranilic acid	524nm	25–300 μgmL−1	(0.9988–0.9996)
2	Rasagilline with tetrachloro-1,4-benzoquinone	535nm	25–350μgmL−1.	(0.9988–0.9996)
3	Rasagilline with 7,7,8,8- tetra cyanoquinodimethane	843 nm	50–500μgmL−1	(0.9988–0.9996)

The formation of products on the reaction of Rasagilline with different solvents involved different mechanisms. The sites of interaction were confirmed by elemental analysis using IR and 1H-NMR spectroscopic methods. The validation of the methods was carried out by accuracy, precision, specificity, linearity, robustness, the limit of detection, and limit of quantitation. No interference was observed from associated usually present in dosage forms. The methods were applied to the determination of Rasagiline in pharmaceutical preparations[5.]

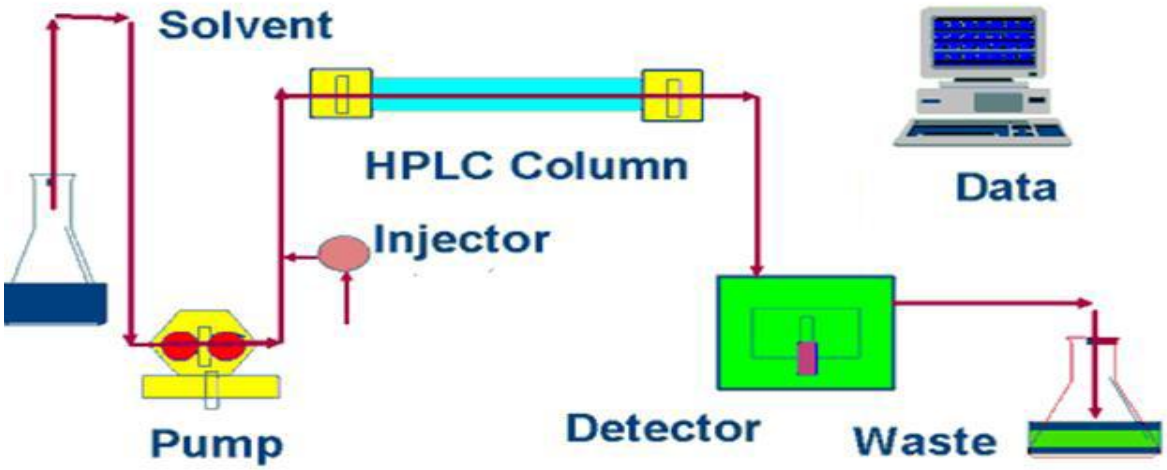
HPTLC METHOD

A simple and sensitive thin-layer chromatographic method was proceeding for analysis of Rasagiline Mesylate in the pharmaceutical dosage form. on silica gel 60 F254 plates the mobile phase butanol:methanol: water 6:1:2(v/v/v)was furnished and compact spots were observed at RF 0.76±0.01. Densitometric analysis was performed at 255 nm. For the specificity of the method, Rasagiline Mesylate was subjected to neutral, hydrolysis, acid-base, photolysis, oxidation, and thermal decomposition, and the peaks for degradation products were well resolved from that of the pure drug. Linear regression analysis shows a good linear relationship between peak area and amount of Rasagiline Mesylate in range(100–300 ng/band). The minimum amount of Rasagiline Mesylate was detected and quantified at 11.15 and 37.11 ng/band, respectively. The method was validated, as per ICH guidelines for accuracy, precision, and robustness. As the method separate the drug from its degradation products effectively, it was considered as stability-indicating. The introduction of the HPTLC method in the pharmaceutical analysis is a major step in quality assurance. As per the observation of Statistical analysis the method is suitable for analysis of Rasagiline Mesylate as the bulk drug and in a pharmaceutical formulation without the interference of excipients. This is a type of stability-indicating assay method, established according to

recommendations of the ICH guidelines. The method is also used for the determination of purity of a drug and it is also used for the analysis of drug and degradation products instability samples obtained during industrial production[6].

HPLC METHOD OF ANALYSIS

HPLC is the presiding separation technique in modern pharmaceutical and biomedical analysis as it gives highly efficient separations and in most cases, it provides high sensitivity. The drugs in complex dosage forms can be analyzed by the HPLC method as it has several advantages like accuracy, precision, specificity, and rapidity. HPLC method development and validation are important roles in the new discovery, development, manufacture of pharmaceutical drugs. The analytical method was developed to test the character of the drug substance or drug product against established acceptance criteria for that characteristic[7].



Isocratic elution technique

estimation of rasagiline mesylate in different plasma matrices (rat, rabbit, and human plasma)was developed and validated by simple, rapid, and sensitive RP-HPLC method. The method was carried out by isocratic elution technique using Kromasil C18 column and mobile phase composition of 10 mM ammonium acetate buffer: acetonitrile (40:60 v/v). In plasma samples, linear detector responses observed in the concentration range of 0.5–20 mg/mL at 265 nm using an ultraviolet detector. No internal standard was used in this method, as the simple protein precipitation method yields 95% recovery of rasagiline mesylate from all plasma matrices. This validation study of this method gives the accuracy, precision, and selectivity in all plasma matrices. This method was performed to find out the drug-protein binding ratio in plasma matrices and determining pharmacokinetic parameters of rasagiline mesylate by non-compartmental analysis, the drug is administered by oral root, and plasma was taken from a rabbit. A mobile phase consisting of aqueous phase (10 mM ammonium acetate) and acetonitrile in the ratio of 40:60 at a flow rate of 1 mL/min was selected as the optimal condition for the developed method. With optimized mobile phase condition, the retention time of RM was found to be 5.72+0.17 min with a maximum tailing factor of 1.09+0.03 across all the plasma matrices. Chromatographic peak parameters obtained for the method across all plasma matrices and three QC levels are presented below.

Table 2: Chromatographic peak parameters for rasagiline mesylate in various plasma matrics

Plasma matrix	QC sample level	Retention time	Tailing factror	Thereotical plates
Rat	LQC (1.5µg/ml)	5.71	Less than 2.00	More than 2000
	MQC(7µg/ml)	5.64	Less than 2.00	More than 2000
	HQC(17µg/ml)	5.78	Less than 2.00	More than 2000
Rabbit	LQC(1.5µg/ml)	5.76	Less than 2.00	More than 2000
	MQC(7µg/ml)	5.77	Less than 2.00	More than 2000
	HQC(17µg/ml)	5.64	Less than 2.00	More than 2000
Human	LQC(1.5µg/ml)	5.78	Less than 2.00	More than 2000
	MQC(7µg/ml)	5.72	Less than 2.00	More than 2000
	HQC(17µg/ml)	5.69	Less than 2.00	More than 2000

The above reports obtained by the RP-HPLC method was described for the qualitative and quantitative analysis of Rasagiline mesylate in rat, rabbit, and human plasma matrices. The developed method was accurate and precise, and recovery of the drug from all plasma matrices is more. The pharmacokinetic study carried in rabbits to determine parameters and proves the applicability of the method to real-time sample analysis. As this method is validated in different plasma matrice[8.]

Gradient elution technique

Method 1

To develop RP-HPLC method for validation and quantitative estimation of the synthesized drug rasagiline hemitartrate in bulk form. Chromatographic separation was carried out by using Agilent TC-C18 (250_4.6mm,5 mm) column at ambient temperature and the mixture of 20mM potassium dihydrogen orthophosphate buffer (pH 7.0): methanol and acetonitrile in the ratio (30:30:40 v/v) as a mobile phase and at a flow rate of 1.0 mL/min, while UV detection at 285 nm. the validation methods linearity, precision, accuracy, ruggedness and robustness, LOD, LOQ were detected as per the ICH guidelines. By using the above parameters the retention time for rasagiline hemitartrate was observed at 4.30min. The method was linear in the range of 10-50 mg/mL. The limit of detection 0.651mg/ml and quantization 1.972 mg/mL . Analytical recovery was found 100.47%. The percentage RSD for precision and accuracy of the method was less than 2%. The correlation coefficient was 0.9952. By the above observation, the developed RP-HPLC method for the synthesized rasagiline hemitartrate was found accurate, precise, and validated statistically. For estimation of rasagiline hemitartrate in bulk forms, the above method can be applied as its simplicity, fastness, and robust nature[9.]

Observation:

Table 3

Sl.no	Validation method	Observation
01	Retention time	4.30 min
02	Linearity	10-50mg/ml
03	LOD	0.651
04	Accuracy	Less than 2%
05	Correlation coefficient	0.9952

Method 2

Rasagiline was extracted by liquid-liquid extraction method and analyzed by reversed-phase high-performance liquid chromatography (HPLC). The separation was performed on a Lichrosphere C18 column (250 x 4.6 mm, 5 µm particle size) at ambient temperature. Using a mixture of acetonitrile and ammonium acetate (pH 5.8) (45:55, v/v) as mobile phase at a flow rate of 1 mL/min. and rasagiline was detected at a wavelength of 265 nm by UV detection. This method was validated as per European Medicine Agency (EMA) guidelines[10]

Method 3

Estimation of rasagilline by RP-HPLC using mobile phase of mixture of acetonitrile and water in the ration of 50:50, adjusted to pH 3.0 ± 0.05 with ortho-phosphoric acid. phenomenex 100 C18 (250 × 4.6 mm, 5 µm) column an ultraviolet detector at chromatographic conditions of ; flow rate 0.8 ml/minute, run time 6.0

minutes, injection volume 50 µl, and detection wavelength 268 nm were maintained during the study at room temperature and all the validation parameters are carried out and all validation parameters observed within permissible range as per ICH guidlines[11].

UPLC METHOD

The key focus of the pharmaceutical or chemical industries is to reduce the cost involved in the development of new drugs and to improve the selectivity, sensitivity, and resolution for their detection. The aim is to be solved by the separation method called UPLC. which is the modified HPLC method with high pressure and small-sized particles (less than 2 µm) used in the column so that the length of the column decreases results in time-saving and reduction in the consumption of solvent. The main principle of UPLC is based on van Deemter statement which narrates the connection between linear velocity and height of the plate. UPLC helpful in the development of the three areas: sensitivity, speed, and resolution. This method is a new and advanced category of the HPLC. which has the same basic principle and methodology as in HPLC, with improved chromatographic performance. UPLC is one of the most important tools in analytical chemistry as it increases the sensitivity, speed, and resolution, of the chromatographic analysis in less time and less solvent consumption and cost involved. The peaks observed through UPLC shows decreased noise and a better signal to noise ratio. It gives sharp and narrow peaks of almost all categories of pharmaceutical drugs. It also useful in the analysis of complex mixtures in less time and the peaks obtained by this method shows more information which is more clear in comparison to the peak observed by HPLC. This method is widely used for the analysis of different pharmaceuticals such as amino acids, peptide mapping, glycans analysis, phenotyping, drug discovery, etc[12.]

Isocratic elution approach

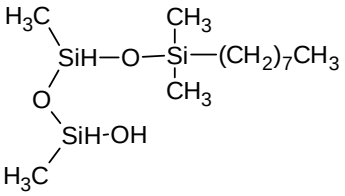
This method was developed as an alternative to the usage of normal-phase chiral LC columns for isomer separation. Here the method is based on an isocratic approach, The advanced chromatographic method was used to enantiomeric separation of rasagiline mesylate and its tartrate salts using a Chiralpak AGP column (50 mm- 2.1 mm, 5 µm) as a stationary phase. And the mixture of ammonium acetate and isopropyl alcohol (90:10, v/v) as the mobile phase at a flow rate of 0.6 mL/min. The detection at a wavelength of 210 nm and the quantification limit for the rasagiline enantiomers were 0.06 and 0.2 µg/mL, respectively. UPLC method is compatible with the MS technique. The successful separation of rasagiline and its enantiomer (rasagiline mesylate and its tartrate salts) was confirmed by determining specific optical rotation values. this method will be applicable for detecting rasagiline enantiomers during the manufacturing processes, and for use in rapid analysis for quality control in the pharmaceutical industry to obtain optically pure pharmaceutical substances. This method was validated in terms of its accuracy, precision, linearity, robustness, ruggedness, specificity, the limit of detection, the limit of quantification, forced degradation. According to ICH Guidelines Q2 (R1). In hyphenated technique (UHPLC-MS) the use of a volatile mobile phase compatible with mass spectrometry (i.e., ammonium acetate and isopropyl alcohol in water) for the determination and separation of the inactive S-enantiomer of rasagiline mesylate from a mixture of the two enantiomers and rasagiline tartrate. The separation parameters were as follows. A Chiralpak AGP column was used as the stationary phase, The column oven temperature was 25 oC, and a mixture of 10 mmol/L ammonium acetate and isopropyl alcohol was used as the mobile phase. the volume of injection was 2.0µL and the detection wavelength at 210 nm. The success of the enantiomeric separation was confirmed by measurements of the optical rotation of compounds[13.]

Table 4: PARAMETERS USE IN HPLC AND UHPLC[14]

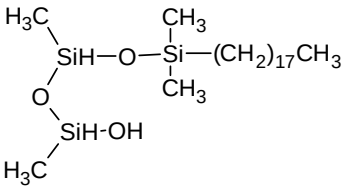
Characteristics	HPLC	UHPLC
Particle size	3-5µm	Less than 2µm
Back pressure	35-40Mpa less	103.5Mpa more
Column	C18, C8	UPLC BEH C 18
Column dimension	150×3.2mm	50×2.1mm
Column temperature	30^0C	65^0C
Analysis time	More	Less
Resolution	Normal	Higher
total run time	10min	1.5min
Run time	3.0ml/min	0.6ml/min

Composition of HPLC and UHPLC columns

1. HPLC columns[15]

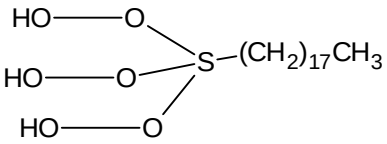


Octylsilane (C8 column)

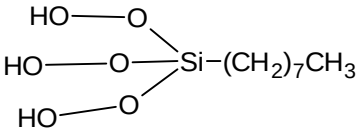


Octadecyalsilane (C18 column)

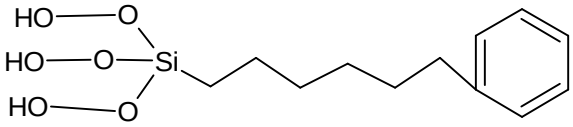
2. **UHPLC columns[16]**



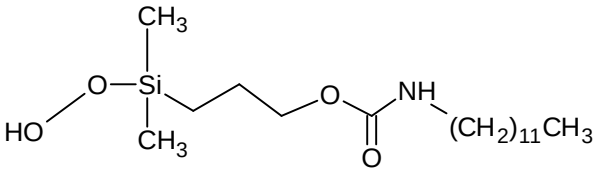
BEH C18 (C18 column)



BEH 8 (C8 column)



BEH Phenyl



BEH shield RP C18

LC-MS METHOD

Gradient elution method

A more sensitive liquid chromatography-tandem mass spectrometric (LC-MS/MS) method was developed for the determination of rasagiline. The plasma was extracted by liquid-liquid extraction with n-hexane–dichloromethane–isopropanol (20:10:1, v/v/v) using as a solvent. pseudoephedrine was used as internal standard (IS) and carried using stationary phase that is Zorbax Extend C18 column (150mm×4.6mm,5_m) with a mobile phase of acetonitrile:5mMammoniumacetate: acetic acid (40:60:0.05, v/v/v) at a flow rate of 0.5 mL/min. The API 4000 triple quadrupole mass analyzer was used in multiple reaction monitoring via positive electrospray ionization (ESI) interface using the transitions m/z 166.0→m/z 148.1 for the internal standard and m/z 172.1→m/z (117.1 + 115.1) for rasagiline. The method shows linear over the concentration range of 0.020–50.0 ng/mL. By using the above parameters the accuracy was within ±6.4% in terms of relative error (RE). The lower limit of quantification (LLOQ) was detected and reproducible at 0.020 ng/mL with acceptable precision and accuracy. The intra- and inter-day precisions were observed less than 11.2% in terms of relative standard deviation (R.S.D.), and The mean extraction-efficiency at three concentrations was 95.6±7.0%, 97.9±3.0%, and 95.3±8.3%. The pharmacokinetic studies were observed on oral administration rasagiline it remains in plasma depends on dose 4 h (1 mg), 6 h (2 mg), and 12 h (5mg). The profiling time was about twice longer than those

reported. This method has advantages of wide linear concentration range, short run time, and simple sample preparation[17].

Table 5

Sl.no	Validation methods	Observation
1	Linearity	0.020–50.0 ng/Ml
2	Accuracy un terms relative error	±6.4%
3	Lower limit of quantification	0.020 ng/mL
4	Inter-and intra day precisions	less than 11.2%

Isocratic elution method

The bio-analytical method was based on liquid-liquid extraction and it has been developed and validated for quantification of Rasagiline in human plasma. This method involved simple isocratic chromatographic conditions and mass spectrometric detection in the positive ionization mode using an API 4000 system. Rasagiline-13C3 mesylate was used as an internal standard for Rasagiline. And stationary phase is Zorbax Eclipse Plus C18 (2.1mm_50 mm,3.5 mm) column was used, Column temperature was set at 45 0C. and 0.1% formic acid: methanol (80:20,v/v)was used as mobile phase. chromatographic separation of analyte was detected with mass spectrometry. The run time was found at 3.0min. This method was validated with the linear range of 52000pg/mL for Rasagiline. The intra-run and inter-run precision values were within 1.3%- 2.9% and 1.6%– 2.2% respectively for Rasagiline. The overall recovery for Rasagiline and Rasagiline-13C3 mesylate analog was 96.9% and 96.7% respectively. This validated method was applied to the bioequivalence and pharmacokinetic study of human volunteers under fasting conditions. The above bio-analytical method is simple, sensitive, selective, rugged, and reproducible. The main advantage of this method, it shows rapid analysis time (3 min), less plasma volume (0.1 mL) used for analysis, suitable internal standard usage. This method was successfully applied in a bioequivalence study to evaluate the plasma concentrations of Rasagiline in healthy human volunteers[18].

ELECTROPHORETIC METHOD

The Pressure-assisted capillary electrophoresis method was carried out for the determination of enantiomeric purity of (R)-rasagiline. In the developed method, 50mMg lycine-HCl buffer pH 2, supplied with 30mM sulfobutylether -cyclodextrin, at 35°C and applying 12 kV in reversed polarity, and –8 m bar pressure (vacuum), short-end injection with -25 m bar for 2 s, was successful for baseline separation of rasagiline enantiomers and observed Rs = 3.5 ± 0.1 in a short time. The method was validated according to ICH guidelines and validated to be linear, precise, and accurate, reliable for the determination of 0.15% S-enantiomer as a chiral impurity in R-rasagiline compound, as well as quantification of the eutomer. The above method was tested by taking commercial tablet formulation. Determination of the structure of diastereomeric associates was done by 1H and 2D NOESY NMR, indicating that the aromatic moiety of the molecule can enter the cyclodextrin cavity. NMR titration and molecular modeling revealed that S-rasagiline formed a more stable inclusion complex with sulfobutylether-cyclodextrin than its antipode, which is in agreement with electrophoretic results. A novel, rapid enantioselective pressure-assisted CE method using SBE- ß-CD as a chiral selector has been established for the determination of enantiomeric purity of RAS for the first time. Applying the optimized conditions 50mM glycine-HCl buffer pH 2.0, supplied with 30 mM SBE-ß-cyclodextrin, at 35°C, applying 12 kV in reversed polarity, and –8 bar pressure (vacuum), short-end injection with -25m bar for 2s, baseline separation of RAS enantiomers was achieved within 8min. Our optimized and validated method can be successfully applied for the quality control of R-RAS. Using NMR and molecular modeling the SBE-ß-CD RAS inclusion complexation was characterized. Different binding affinities of the individual enantiomers towards SBE- ß -CD were identified with both NMR and molecular modeling, in favor of the S enantiomer, which is in accordance with the observed EMO. The secondary interactions with smaller energies have a bigger role in the enantiomer recognition mechanism. In the above procedure combined use of CE, nuclear magnetic resonance spectroscopy, and molecular modeling calculations help to gain a deeper understanding of the chiral recognition mechanism of CD chiral selectors[19].

FLURIMETRIC DETECTION

Rasagiline mesylate contains terminal alkyne moiety that was observed in biological samples with azide-alkyne cycloaddition reaction (CuAAC reaction). In the fluorimetric study, dansyl azide introduces as a fluorescence labeling reagent. Rasagiline mesylate is made to react with dansyl azide (DNS-AZ) in presence of copper and sodium ascorbates catalyst which forms a stable 1,2,3-triazole derivative which is determined by HPLC with fluorescence detection. The fluorescence methodology was optimized for sensitive and selective determination of rasagiline in rat plasma, with selegiline as an internal standard. The method allowed an accurate and precise determination of RSM in the linearity range 0.50–100 ng mL−1 with a detection limit of 0.16 ng mL−1 for RSM in rat plasma[20.]

CONCLUSION

This review gives information regarding various stages and methods involved in the development and validation of Rasagilline by various analytical methods such as UV-VIS, High-Performance Liquid Chromatography (HPLC), Ultra Pressure liquid chromatography (UHPLC), LC-MS, Electrophoresis method, and Fluorimetric determination which are performed as per ICH Guidelines and covers all the performance characteristics like validation, Accuracy, precision, specificity, linearity, range and limit of detection, the limit of quantification, robustness and system suitability testing all methods are found in the permissible limit.

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