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Research Article

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Determination and Validation of trace levels of Methylamine and ethylmethylamine in Rivastigmine Tartarate drug substance by ion chromatographic technique

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Abstract

A sensitive ionchromatgraphic method was developed and optimized for the determination of methylamine and ethylmethylamine in rivastigimine tartarate drug substance, which in lower limits may act as potential impurities and cause undesirable side products. The method was developed to enhance the detection by this technique and minimizing the run time which uses 20 minutes. To prove the performance characteristic of the given method, validation of this technique performed as per the ICH guidelines requirements for the parameters such as selectivity, sensitivity, LOD, LOQ, linearity, precision, robustness and accuracy.

Keywords: drug substance, ICH guidelines, selectivity, sensitivity, LOD, LOQ.

Introduction

Rivastigmine Tartrate, the chemical (S)-3-(1-(dimethylamino)ethyl)phenyl ethyl(methyl) carbamate (2R, 3R)-2, 3-dihydroxysuccinate (**figure 1**), with a carbamate in its structure, is the first FDA approved drug for the treatment of mild to moderate dementia of the Alzheimer's type[1-4] and dementia related to Parkinson's disease[5]. It was believed to work by blocking the acetylcholine esterase (the enzyme) responsible for its degradation and butyryl-cholinesterase, (enzyme) responsible for hydrolysis of acetylcholinestarase, in so doing increasing both the levels and duration of act of the neurotransmitter acetylcholine [6-7]. Rivastigmine in its action appears to have clear effects in patients, showing further hostile course of disease, such as those with a younger age of beginning or a poor nutritional rank or those experiencing symptoms of nausea and vomiting. The potential of rivastigmine drug substance against Alzheimer's disease deserve to be in the list of "Blockbuster Drug".

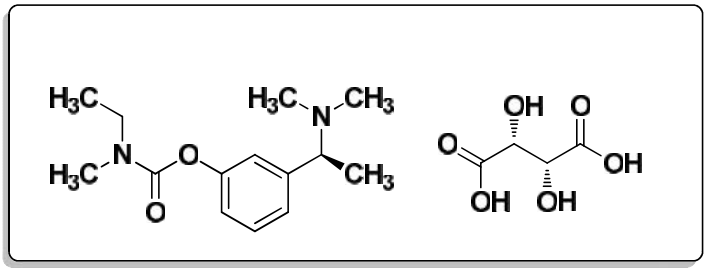

Figure 1: Structure of Rivastigmine Tartarate

During the process preparation of rivastigmine tartarate two of the genotoxic impurities *i.e*. methylamine and ethylmethylamine were used in the basic intermediary stages[8]. These reagents may be present in the final drug substances as impurities which were believed as active reactive impurities with undesired toxicities, including genotoxicity and carcinogenicity. Such impurities should be controlled in the drug which has to be used daily and should not exceed the daily limit dose, which fall at low in ppm levels[9]. For this purpose, a simple sensitive analytical technique should be made available which allows the determination of low level chemical entities in ppm levels. Fewer reports available for the determination of methylamine and ethylmethylamine, using techniques of HPLC and GC methods for their determination[10-11].

Several reports reported for their individual identification [12-14]. These types of chemical entities can be analyzed using ion chromatographic technique. A method need to be finalized where dual chemical entities identified and characterized. These two impurities were estimated in trace levels using ion chromatographic technique. Where the technique was adopted due to its sensitivity for the detection in trace levels uses eco-friendly chemicals and cheaper compared to other techniques like high performance liquid chromatographic and gas chromatographic technique.

Materials and Methods

Chemicals and reagents

Methylamine and ethylmethylamine reagents which were used for reference standards, AR grade Nitric acid was procured from E. Merck, India. Water was distilled and purified with Millipore system (Millipore Milford, MA USA). The known related substances of Rivastigmine Tartrate were prepared at Aurobindo Pharma Ltd. Research Centre, India were use for studies.

Instrumentation:

Ionchromatography:

An ion chromatography instrument employed in this work was with version Metrohm 733 IC Separation center with conductometric detector, Metrohm 732 IC module, Metrohm 709 IC Pump, Metrohm 813 compact auto-sampler and 762 interface or equivalent with Metrohm IC Net 2.3 or equivalent data handling system.

The sample and standard analysis were carried out on METROSEP CATION C4-250 (Metrohm, 250mm x 4.0mm, 5µm particle size) column packed with silica gel with carboxyl groups as stationary phase at ambient temperature. The mobile phase was delivered in an isocratic mode at a flow rate of 1.0 mL/min. The detector was operated in conductivity mode and analogue range of the detector was set at scale range 2mS/cm and Full Scale10µS/cm .The injection volume was 20µl. The retention times were calculated for all the three entities. The total run time was 20 min.

Preparation of Solutions

Preparation of Mobile Phase

Carefully transfer 6.3 mL of nitric acid into 100 mL volumetric flask containing about 50 mL water and makeup the same with water. Transfer 5.0 mL of above prepared nitric acid solution into 1000 mL volumetric flask and dilute the same with water. Filter through 0.45µ finer porosity membrane filter. Use this solution as mobile Phase.

Preparation of diluents

Water is used as diluent.

Preparation of standard stock solution

Accurately weigh and transfer about 110 mg each of methylamine and ethylmethylamine reference standards into 200 mL clean dry volumetric flask and

add 100 mL of diluent and sonicate to dissolve. Make up to volume with diluent. Dilute 10 mL of above solution to 100 mL using diluent. Further dilute 4 mL of above solution to 100 mL using diluent. Filter through 0.45µ finer porosity membrane filter.

Sample Solution

Accurately weigh and transfer about 150 mg of sample into 100 mL clean dry volumetric flask and add 50 mL of diluent, sonicate to dissolve. Make up to volume with diluent and filter through 0.45 µ finer porosity membrane filter.

Chromatographic conditions:

Column used was METROSEP CATION C4-250(6.1050.430), 250 x 4.0mm, 5µ, flow rate of 1.0mL/min and injection volume of 20µl. Data acquisition time was 20 min and for detection non suppressor with conductivity detection was used.

Evaluation of system suitability:

Separately inject 20 µl of standard solution, six times into the chromatograph, record the chromatograms and measure the peak areas. RSD for peak areas of six injections of the standard solution is not more than 5.0% respectively.

Procedure

Inject 20 µl of diluent into the chromatograph and record the chromatogram. Inject 20 µl of sample solution into the chromatograph, record the chromatogram and measure peak areas.

Examine the diluent chromatogram and no interference peak should be observed at the retention times of Methylamine and Ethylmethylamine Integrate peak due to Methylamine and Ethylmethylamine only.

Results and Discussion

Method Development

The low molecular weight organic bases have a greater affinity and can easily bind or exchange with ion chromatography column stationary phase, which helps to determine the ionisable analytes in the drug substances. The determination of low molecular mass amines by IC was typically achieved by using either silica or resin based cation exchange column.

Initial trials attempted using the **METROSEP CATION** C4-250 (Metrohm, 250mm x 4.0mm) column, where all the standards methylamine and ethylmethylamine were injected. Mobile phase used was 2 mM of nitric acid, but at this buffer strength, peaks were retained and peaks are distinctive in shape. In another trial buffer strength increased as well with introducing of organic solvent. Where buffer strength used was 10 mM of nitric acid. The results yielded the separation of methylamine and ethylmethylamine within 5 mins of retention time, but the elution of analytes were almost close to each other and the separation of two analytes were not so satisfactory. The above obtained results prompted us to optimize this condition for better resolution of peaks.

Further trial attempted using buffer strength as 2 mM of nitric acid and the mobile phase ratio fixed to (buffer: acetonitrile) as 85: 15 v/v in ratio. The results yielded the separation of all two chemical substances and merging of methylamine and ethylmethylamine was observed. Later another trial attempted using buffer strength as 5 mM of nitric acid used as mobile phase. By increasing the nitric acid 2mM to 5 mM and removing the acetonitrile, separation of two analytes with good resolution was observed. Later to know the interferences of other analytes, a specification trial attempted with all the known impurities available. But no interference of other analytes was observed.

Method Validation

In order to determine the methylamine and ethylmethylamine in rivastigimine tartarate drug substance, the method was validated as per the ICH guidelines. Individually in terms of specificity, LOD, LOQ, linearity, accuracy and precision of sample solution.

Specificity

To prove the selectivity of the method, it is necessary to evaluate a retention time of each impurities present in the drug substances. To identify analyte, each solution was prepared individually the retention time of each analyst, each solution was prepared as per the methodology. Further the sample solution was prepared by spiking known related substances of rivastigimine tartarate drug substance at about 0.06 %w/w and injected as per procedure and conform the no co-elution of peaks from the sample matrix. The chromatogram of each analyte clearly shows that the methylamine and ethylmethylamine peaks were well resolved from that of rivastigimine tartarate drug substance, related substance of the rivastigimine tartarate and blank solution which indicated that the method is selective for determination of methylamine and ethylmethylamine in rivastigimine tartarate.

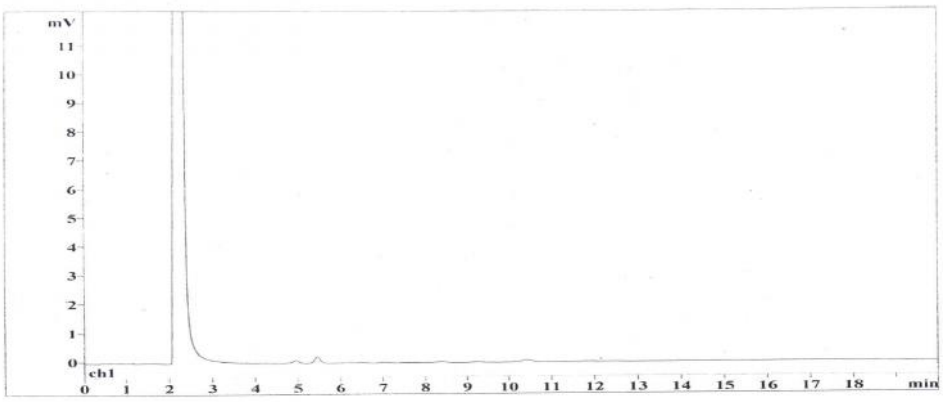

Figure 2: Diluent Chromatogram

An overlay chromatogram of diluent, standard solution and sample solution spiked with known amount related impurities of saxagliptin monohydrate Figure 2, 3& 4.

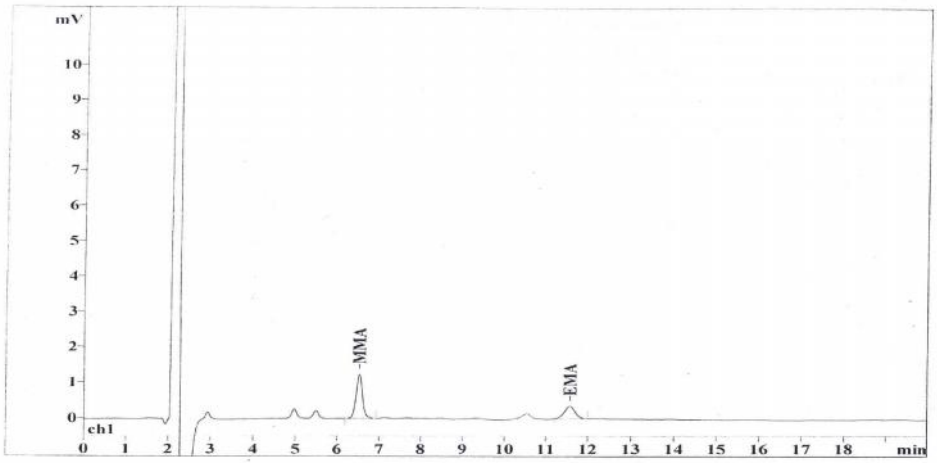

Figure 3: Chromatogram of methylamine and ethylmethylamine standards.

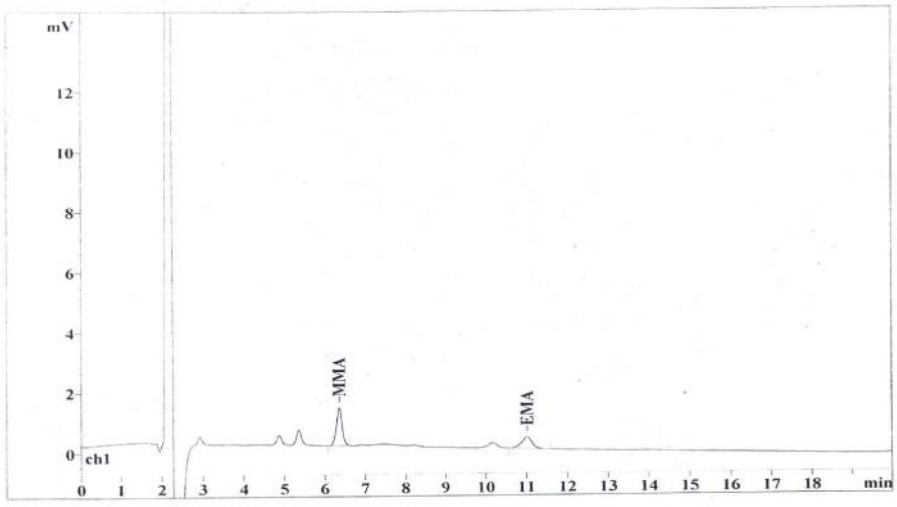

Figure 4: Chromatogram rivastigimine tartarate drug substance spiked with all known related substances including methylamine and ethylmethylamine spiked sample.

Table 1: Specificity values were clubbed with method precision data of monomethylamine and ethylmethylamine.

S. No.	Sample		Monomethylamine (ppm)	Ethylmethylamine (ppm)
1	Method Precision-1		615	592
2	Method Precision-2		586	582
3	Method Precision-3		612	610
4	Method Precision-4		603	641
5	Method Precision-5		609	597
6	Method Precision-6		605	588
7	Specificity-1		579	595
8	Specificity-2		585	588
9	Specificity-3		581	605
Method Precision		**Mean**	605	602
		SD	10.296	21.4
		% RSD	1.7	3.6
Specificity		**Mean**	582	596
		SD	3.055	8.544
		% RSD	0.5	1.4
Over all Mean			597	600
Over all Standard Deviation			14.307	17.733
Over all % RSD			2.4	3.0

Note: *Acceptance Criteria: % Relative standard deviation, when the method precision value clubbed with specificity sample value should not be more than 10.0.*

No peaks were observed at the retention times of the analytes in the chromatograms. From the above data it can be inferred that in the presence of all the impurities also there is no interference in the retention times of methylamine and ethylmethylamine analytes. When the method precision value clubbed with specificity sample, the overall % Relative standard deviation value is found to be below the acceptance criteria.

LOD and LOQ.

The sensitivity of the method was evaluated by constructing a linearity curve. The solutions of different concentrations of methylamine and ethylmethylamine solutions were prepared from a lower concentration level of 0.103 ppm to a higher concentration level of 1.284 ppm and 0.098 ppm to a higher concentration level of 1.230 ppm. The slope (S) and residual standard deviation (SD) were determined from the linearity curve. By using a slope (S) and residual standard deviation (SD) the limit of quantification and limit of detection of the method was arrived.

The formula used for the determination of LOQ and LOD were 10 x STEYX/SLOPE and 3.3 x STEYX/SLOPE respectively. The predicted LOQ and LOD levels for methylamine and ethylmethylamine were LOD: 0.051 µg/ml LOQ: 0.154 µg/ml and LOD:0.061 µg/ml LOQ: 0.185 µg/ml respectively.

To prove the predicted levels of LOQ and LOD values are precision and these levels can be easily quantify in the sample without any ambiguity. The solutions were prepared at the predicted concentration of LOD and LOQ levels, and analyzed for six times, and the percentage relative standard deviation was found to be LOD(MMA):14.6, LOQ(EMA): 5.3 and LOD(EMA):13.5, LOQ(EMA):5.4 respectively. The data of six-replicated injection for LOQ and LOD is tabulated in the Table 2.

Table 2: Precision data of **LOD** and **LOQ** of methylamine and ethylmethylamine.

Injection ID	Methylamine		Ethylmethylamine	
	LOD (Area Count)	**LOQ (Area Count)**	**LOD (Area Count)**	**LOQ (Area Count)**
1	0.656	1.619	0.720	1.846
2	0.610	1.752	0.669	1.842
3	0.760	1.731	0.641	1.760
4	0.488	1.762	0.850	1.691
5	0.614	1.550	0.901	1.646
6	0.702	1.615	0.769	1.636
Mean	0.638	1.672	0.758	1.737
SD	14.6	5.3	13.5	5.4
% RSD	0.053	0.160	0.062	0.185
Concentration(µg/mL)	**35**	**107**	**41**	**123**

Linearity:

The detector response was established by preparing a series of diluted solution of methylamine and ethylmethylamine as per the methodology from 0.103 µg /mL to a higher concentration level of 1.284 µg /mL and 0.098 µg /mL to a higher concentration level of 1.230 µg/ml. Each solution was injected into the ion chromatography and measure the response of methylamine and ethylmethylamine and concentration of the solutions. From the area response of the analytes and concentration of the linear regression line plotted was constructed. From the linear regression line, the correlation coefficient of the regression line was found to be 0.9996 and 0.9988 respectively. The statistical analysis of linear regression line was evaluated and is summarized in table 3 & 4 and linearity plot of concentration of methylamine and ethylmethylamine Vs area response is shown in the figure 5 & 6.

Table 3: Linearity data shows the concentration of methylamine and area response of each concentration

S. No.	Concentration (µg/mL)	Area (Area Counts)
1	0.103	1.528
2	0.205	2.675
3	0.308	4.118
4	0.411	5.638
5	0.514	7.368
6	0.616	8.666
7	0.822	11.306
8	1.027	14.445
9	1.284	17.422
Slope		13.776
Intercept		0.032
STEYX		0.216
Correlation Coeffecient		0.9993

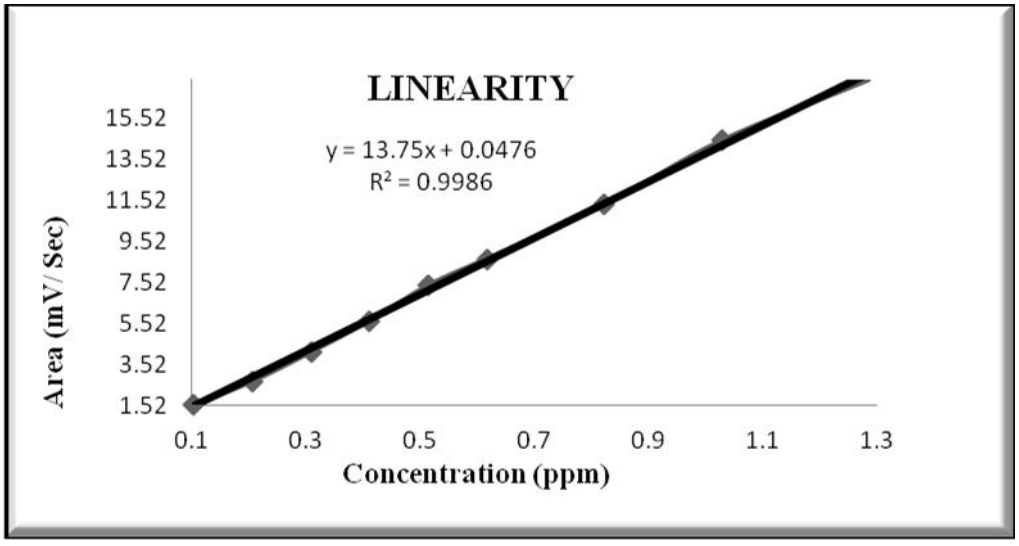

Figure 5: Linearity plot of Concentration of Monomethylamine acid Vs Area response.

Table 4: Linearity data showing the concentration of Ethylmethylamine and area response of each concentration

S. No.	Concentration (µg/mL)	Area (Area Counts)
1	0.098	0.693
2	0.197	1.457
3	0.295	2.120
4	0.394	2.936
5	0.492	3.398
6	0.590	4.457
7	0.787	5.937
8	0.984	7.474
9	1.230	8.977
Slope		13.776
Intercept		0.032
STEYX		0.216
Correlation Coeffecient		0.9993

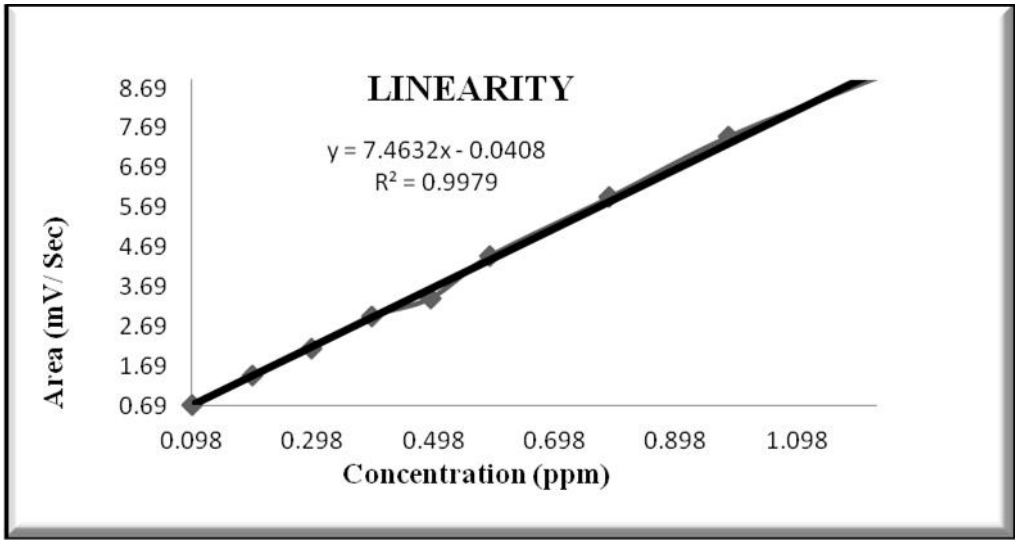

Figure 6.: Linearity plot of Concentration of Ethylmethylamine Vs Area response.

Accuracy

The recovery or accuracy of the method was tested by adding the methylamine and ethylmethylamine to the rivastigmine tartrate drug substance at three different concentration levels. These concentrations were prepared by adding methylamine and ethylmethylamine to the rivastigmine tartrate about LOQ Level-(MMA 100 ppm), (EMA 123 ppm), 333ppm,666ppm and 999ppm Sample solutions were prepared in triplicate for each concentration and injected into the IC system and calculate the amount of methylamine and ethylmethylamine present. The mean recovery was found to be for LOQ Level (107 ppm and 123 ppm) 101.9%(MMA), 100.8%(EMA) and mean recovery was found to be for 50%,100% and 150% of specification level(666ppm) is 89.7%(MMA), 89.3%(MMA), 88.7%(MMA) and90.3%(EMA), 99.1%(EMA), 88.2%(EMA) for methylamine and ethylmethylamine respectively. The results are summarized in table 5 & 6.

Table 5: The recovery data of methylamine in rivastigmine tartrate.

Concentration/Sample ID	Amount Added (ppm)	Amount Recovered (ppm)	Recovery (%)	Statistical Analysis	
LOQ Level Sample-1	102	107	104.9	**Mean**	101.9
LOQ Level Sample -2	103	102	99.0	**SD**	2.950
LOQ Level Sample -3	103	105	101.9	**RSD %**	2.9
50% level Sample -1	338	309	91.4	**Mean**	89.7
50% level Sample -2	333	295	88.6	**SD**	0.5
50% level Sample -3	336	299	89.0	**% RSD**	0.6
100% level Sample -1	675	594	88.0	**Mean**	89.3
100% level Sample -2	672	604	89.9	**SD**	1. 1
100% level Sample -3	672	604	89.7	**% RSD**	1.2
150% level Sample -1	1008	904	88.5	**Mean**	88.7
150% level Sample -2	1013	896	88.0	**SD**	0.9
150% level Sample -3	1007	886	89.2	**% RSD**	1.0
Overall Statistical Analysis					
Mean		89.2			
SD		1.11			
% RSD		1.2			
95 % Confidence Interval		±0.9			

Table 6.: The recovery data of ethylmethylamine in rivastigmine tartrate.

Concentration/Sample ID	Amount Added (ppm)	Amount Recovered (ppm)	Recovery (%)	Statistical Analysis	
LOQ Level Sample-1	122	126	103.3	**Mean**	100.8
LOQ Level Sample -2	124	114	91.9	**SD**	7.991
LOQ Level Sample -3	124	133	107.3	**RSD %**	3.7
50% level Sample -1	332	296	89.2	**Mean**	90.3
50% level Sample -2	328	306	93.3	**SD**	3.1
50% level Sample -3	331	293	88.5	**% RSD**	3.7
100% level Sample -1	665	630	94.7	**Mean**	91.1
100% level Sample -2	662	593	89.6	**SD**	3.1
100% level Sample -3	662	590	89.1	**% RSD**	3.4
150% level Sample -1	992	895	90.2	**Mean**	88.2
150% level Sample -2	998	890	89.2	**SD**	2.6
150% level Sample -3	991	845	85.3	**% RSD**	3.0
Overall Statistical Analysis					
Mean		89.9			
SD		2.73			
% RSD		3.0			
95 % Confidence Interval		±2. 1			

Precision:

System Precision, method precision and intermediate precision were performed using methylamine and ethylmethylamine standard solution was prepared as per the methodology. In system precision methylamine and ethylmethylamine solution was injected into the system for six replications and calculated the percentage relative standard deviation of replicate injections (**Table 7**).

Table 7: The System precision data for Methylamine and Ethylmethylamine in Rivastigmine tartrate drug substances and its statistical data.

Sample	Area(mV*sec)	
	Methylamine	Ethylmethylamine
1	13.262	6.814
2	14.004	7.155
3	14.323	7.368
4	14.285	7.000
5	14.401	7.452
6	14.401	7.503
Mean	14.113	7.215
SD	0.442	0.273
% RSD	3.1	3.8
95% Confidence Interval (CI)	±0.5	±0.3

In method precision, the sample solution of rivastigmine tartarate solution of the same batch substance was prepared in six times as per methodology. The six preparations of the sample solutions were separately injected to the chromatogram and evaluate the repeatability to the test method by calculating the content of the methylamine and ethylmethylamine in the sample solution for the six preparations and the relative standard deviation. The amount of methylamine and ethylmethylamine and its percentage relative deviation were tabulated in **table 8**.

Table 8. The method precision data for Methylamine and Ethylmethylamine in Rivastigmine tartrate drug substances and its statistical data.

Sample	Contents (ppm)	
	Methylamine	Ethylmethylamine
1	615	592
2	586	582
3	612	610
4	603	641
5	609	597
6	605	588
Mean	605	602
SD	10.296	21.472
% RSD	1.7	3.6
95% Confidence Interval (CI)	±10.8	±22.5

Conclusion

A rapid and sensitive ion chromatography method was developed, optimized and validated for the determination of methylamine and ethylmethylamine. The results of various validation parameters demonstrated that the method is specific, linear, precise and accurate in rivastigmine tartarate drug substance.

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