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Spectrophotometric Method for the Determination of Trace Level Formaldehyde in Rivastigmine Tartrate Drug Substance

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ABSTRACT

To develop a fast and sensitive UV spectrophotometric method for the quantitative estimation of Formaldehyde in Rivastigmine tartrate drug substances and validate as per ICH guidelines. The method was based upon the observation, that a characteristic colour results upon addition of solution of Pentane-2,4-dione , also known as acetylacetone . In acetic acid and ammonium acetate buffer condition, acetylacetone and formaldehyde react to form dimethyl pyridine. Dimethyl pyridine is slightly yellow and its absorption maximum in aqueous solution is λ 420 nm in Rivastigmine tartrate drug substance. The developed method resulted in Formaldehyde exhibiting linearity in the range 0.975 to 234 µg/g. The Intraday and interday precision is exemplified by relative standard deviation of 0.562 % and 0.757%. Percentage Mean recovery was found to be in the range of 98-101%, during accuracy studies. The limit of detection (LOD) and limit of quantitiation (LOQ) were found to be 1.3 µg/g and 3.9 µg/g respectively.The present work was aimed to develop a visible spectrophotometric method, which is simple, sensitive, accurate and cost effective to evaluate the quality of the bulk drugs.

Keywords: UV Spectrophotometry, Formaldehyde, Rivastigmine Tartrate, Method development, Validation.

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INTRODUCTION

Rivastigmine tartrate is a reversible cholinesterase inhibitor and is known chemically as (S)-N-Ethyl-N-methyl-3-[1-(dimethyl amino) ethyl]- phenyl carbamate hydrogen-(2R,3R)- tartrate (Figure.1), a carbamate inhibitor of acetylcholinestersae is used for the treatment of mild to moderate Alzheimer's disease in adult [1-2]. Exelon helps to slow down the mental decline that happens in people with Alzheimer's disease and it helps to improve the ability to cope with everyday activities. Alzheimer's disease is a progressive neuro-dogenerative disorder characterized by loss of short-term memory and immediate recall and decline in other cognitive functions such as attention [3-4]. Formaldehyde was used in the preparation of one of the intermediate in Rivastigmine tartrate. Formaldehyde has reported that may causes allergies and carcinogenic when traces are involved in the drug substances. Therefore the quantification of this impurity is essential. The novelty of the developed method was that the reagent used are easily available and the mechanisms of reactions of the reagent are already well established. The method was based upon the observation, that a characteristic colour results upon addition of solution of acetylacetone. In acetic acid and ammonium acetate buffer condition, acetylacetone and formaldehyde react to form dimethyl pyridine. Literature survey reveals many analytical methods include Capilllary Electrophoresis, HPLC, Gas Chromatography, ion-chromatography, and other derivatization UV Spectrophotometry method for the determination of formaldehyde in pollutants and pharmaceutical drug samples [5-9]. Therefore, an attempt was made to develop a simple and rapid derivatization approach of spectrophotometric method for the determination of formaldehyde in pharmaceutical drugs.

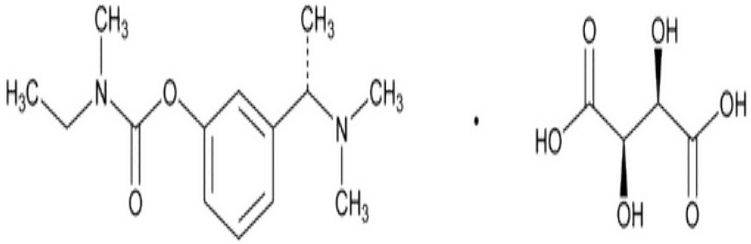

Figure 1: Chemical Structure of Rivastigmine Tartrate.

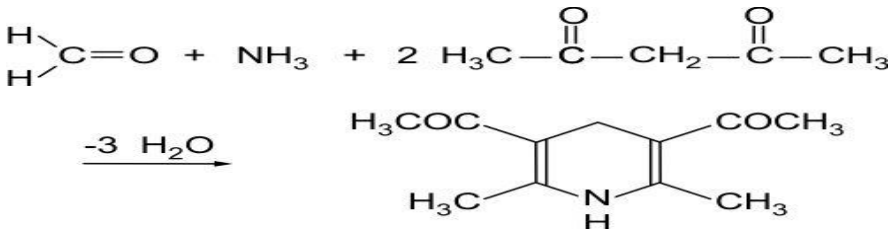

Figure 2: Basic reaction involved for the colour development

MATERIALS AND METHOD

Instrument

A double beam UV-visible spectrophotometer (Shimadzu, model 1800) having two matched quartz cells with 1 cm light path and loaded with UV probe software (version 2.41) was used for recording of spectra and measuring absorbance.

Chemicals and Reagents

Pure drug Rivastigmine tartrate was provided by our APL Research Centre-II. (A Division of Aurobindo Pharma Ltd). All the reagents and chemicals used were of analytical grade from Merck Chemicals, India.

Methods

Preparation of Solutions

Reagent solution

Dissolve about 15g of Ammonium acetate in a 100ml volumetic flask containing about 50ml of water and 0.3ml of acetic acid. Then, add 0.2 ml acetyl acetone and makeupto volume with water.

Standard solution

Prepare 5 µg/ml formaldehyde in water, transfer 15.6 ml of this solution into a 50 ml volumetric flask containing 20 ml of water, dissolve completely and add 5 ml of reagent solution make upto volume with water

Sample solution

Transfer about 1.0 g of sample into a 50 ml volumetric flask, contatining 30 ml of water, dissolve completely and add 5 ml of reagent solution make upto volume with water. Figure 3.

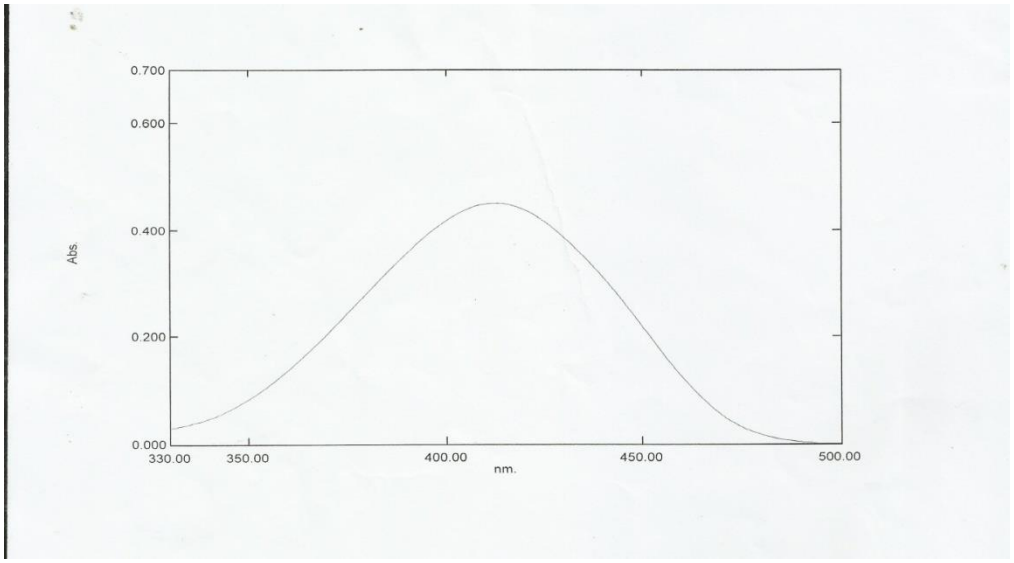

Figure. 3: UV Spectrum of derivatised formaldehyde

Blank solution

Prepare blank solution as per above procedure without sample addition.

Procedure

Heat all the solutions viz., blank, sample and standard at 40±2°C on a water bath for 30 minutes. Then filter through 0.45um PTFE membrane filters. The developed slightly yellow coloured chromogen of standard and sample solutions against the reagent blank was scanned between 400 nm to 600 nm and the absorbance maxima at λ 420 nm

Spectral Characteristics

Standard solutions of derivatized formaldehyde at different concentrations level were prepared. Calibration curve was constructed by plotting the concentration level versus corresponding absorbance at 420nm. The results show an excellent correlation between absorbance and concentration level of formaldehyde within the concentration range 0.975 to 234 µg/g show good agreement with Beer's law. Figure 4

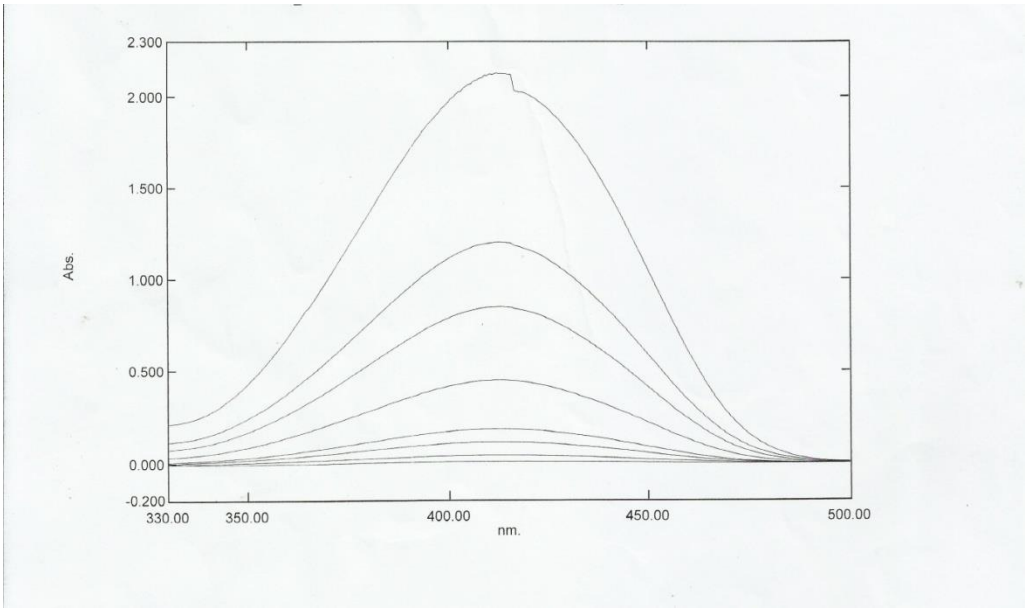

Figure 4: Overlay spectrum of derivatised formaldehyde at different concentrations

RESULTS AND DISCUSSIONS

Method development

The most commonly used method for the determination of free formaldehyde in drug substance material is the Pentane-2, 4-dione method, also known as acetylacetone method. In acetic acid and ammonium acetate buffer condition, acetylacetone and formaldehyde react to formdimethyl pyridine. Dimethyl pyridine is slightly yellow and its absorption maximum in aqueous solution is 420 nm when heating at 40°c±2°c for 30 min and cool the solutions to room temp then record the

absorbance at 420 nm. The intensity of the colour of the aqueous solution is proportional to the formaldehyde concentration. This is the basis to determine the content of free formaldehyde. We measured the formaldehyde content in drug substance low levels. On the basis of trail and error method colour reagent is prepared

Method validation

Validation of the analytical method is the process that establishes by laboratory studies in which the performance characteristics of the method meet the requirements for the intended analytical application. UV spectrophotometric method developed was validated according to International Conference on Harmonization (ICH) guidelines[10] for validation of analytical procedures. The method was validated for the parameters like linearity, accuracy, system precision, intra-day precision, inter-day precision/ intermediate precision/ ruggedness, robustness, limit of detection (LOD) and limit of quantitiation (LOQ).

Precision

System precision

Six replicate recording of absorbance at 420 nm of standard solution at working concentration showed % RSD (Relative Standard Deviation) less than 1 concerning absorbance for the formaldehyde, which indicates the acceptable reproducibility and thereby the precision of the system. System precision results are tabulated in (Table 1).

Table 1: System precision results of Formaldehyde.

N	Absorbance
1	0.390
2	0.394
3	0.391
4	0.391
5	0.399
6	0.390
Mean	0.393
SD^	0.004
%RSD*	0.892

Note:-^ Standard deviation

* Relative standard deviation

Method precision

Method precision was determined by performing content of formaldehydeby spiking with known concentration of formaldehyde in Rivastigmine tartrate drug substance under the tests of (i) repeatability (Intra day precision) and (ii) Intermediate precision (Inter day precision) performed during 3 consecutive days by three different analysts, at working concentration.

Repeatability (Intra day precision)

Six consecutive recording of absorbance at 420 nm of the formaldehyde from the same homogeneous mixture at working concentration showed % RSD less than 1, which indicate that the method developed is method precise by the test of repeatability and hence can be understood that the method gives consistently reproducible results (Table 2).

Table 2: Intra day precision results of Formaldehyde (78 µg/g) spiked in Rivastigmine Tartrate drug substance.

N	Formaldehyde Content(µg/g)
1	77.60
2	78.81
3	78.27
4	78.09
5	78.19
6	78.69
Mean	78.28
SD	0.44
%RSD	0.562

Intermediate Precision (Inter day precision / Ruggedness)

Six consecutive recording of absorbance at 420 nm of the formaldehyde from the same homogeneous mixture at working concentration on three consecutive days by three different analysts, showed % RSD less than 1 within and between days, which indicate the method developed is inter day precise / rugged (Table 3).

Table 3: Inter day precision results of Formaldehyde (78 µg/g)spiked in Rivastigmine Tartrate drug substance.

N	Formaldehyde Content(µg/g)		
	Day 1	Day 2	Day 3
1	76.92	77.36	77.31
2	77.93	78.89	77.12
3	77.08	78.76	78.11
4	77.26	78.94	78.05
5	78.14	77.91	78.74
6	78.11	78.28	78.10
Mean	77.57	78.36	77.91
SD	0.55	0.63	0.59
% RSD	0.709	0.804	0.757

Linearity

Standard solutions of derivatized formaldehyde at different concentrations level 0.975 to 234 µg/g were prepared. Calibration curve was constructed by plotting the concentration level of formaldehyde versus corresponding absorbance at 420nm. The results show an excellent

correlation between absorbance and concentration level of formaldehydewithin the concentration range (0.975-234 µg/g) are given in (Table 4)**.** The correlation coefficients were greater than 0.999, which meet the method validation acceptance criteria and hence the method is said to be linear in the range of 0.975-234 µg/g

Table 4: Calibration data for Formaldehydestandard.

N	Formaldehyde standard (µg/g)	Absorbance
1	0.98	0.004
2	3.90	0.017
3	15.60	0.074
4	39.0	0.180
5	78.0	0.392
6	117.0	0.582
7	234.0	1.176
Regression Equation	y= 0.005, x= 0.005	
Correlation Coefficient(r^2)	0.999	

Accuracy

Accuracy was determined by means of recovery experiments, by the determination of % mean recovery of sample at three different levels (LOQ-150%). At each level, three determinations were performed. Percent mean recovery was calculated as shown in (Table 5). The accepted limits of recovery are 98% - 101% and all observed data are within the required range which indicates good recovery values and hence the accuracy of the method developed.

Table 5: Recovery results from spiking of Rivastigmine Tartrate drug substance with Formaldehyde.

Accuracy (Average of triplicates)	Level-I (LOQ)	Level-II (100%)	Level-III (150%)
Added(µg/g)	3.90	78.0	117.0
Found(µg/g)	3.87	77.47	116.81
Recovery(%)	99.31	99.33	99.84
RSD(%)	0.39	0.73	0.43

Robustness

The robustness of an analytical method is a measure of its capacity to remain unaffected by small but deliberate variations in method parameters and provides an indication of its reliability during normal usage. It is concluded that the method is robust as it is found that the % RSD is less than 2 for the formaldehyde content despite deliberate variations done concerning compositions of colored reagent and solvents.

Sensitivity

The sensitivity of measurement of formaldehdye content by use of the proposed method was

estimated in terms of the limit of quantitation (LOQ), limit of detection (LOD). The limit of detection (LOD) and limit of quantitiation (LOQ) were found to be 1.3 µg/g and 3.9 µg/g respectively. Optical characteristics results are summarized in (Table 6)

Table 6: Optical characteristics of Formaldehyde content in Rivastigmine Tartrate drug substance.

Parameters	Results
Detection wavelength(nm)	420
Beer's law limits (µg/g)	0.975– 234
Regression equation (y = mx+c)	y = 0.005x – 0.005
Correlation coefficient(r^2)	0.999
LOQ (µg/g)	3.9
LOD (µg/g)	1.3

CONCLUSION

A cheap and a rapid UV spectrophotometric method was developed and validated for the quantitative estimation of formaldehyde in Rivastigmine tartrate drug substance as per ICH guidelines. The developed method resulted in formaldehyde exhibiting linearity in the range 0.975 to 234µg/g. The Intraday and inter day precision is exemplified by relative standard deviation of 0.562 % and 0.757%. Percentage Mean recovery was found to be in the range of 98- 101%, during accuracy studies. The limit of detection (LOD) and limit of quantitiation (LOQ) were found to be 1.3 µg/g and 3.9 µg/g respectively. Accordingly it is concluded that the developed UV spectrophotometric method is accurate, precise, linear, rugged and robust and therefore the method can be used for the routine analysis of formaldehyde content in Rivastigmine tartrate drug substances.

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