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A NOVEL SPECTROFLUORIMETRIC METHOD FOR THE DETERMINATION OF MEMANTINE HYDROCLORIDE IN BULK AND PHARMACEUTICAL FORMULATION

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Keywords:

Memantine hydrochloride, spectrofluorimetric method, analytical assay method, 1, 2 - Naphthoquinone - 4- sulphonate

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ABSTRACT: The author selected and developed a new simple, sensitive and reproducible spectrofluorimetric method and validated for the determination of Memantine Hydrochloride in bulk as well as pharmaceutical formulations depending on condensation of Memantine hydrochloride with 1, 2 - Naphthoquinone -4- sulphonate (NQS) in an alkaline medium to become a product with orange color. The spectrofluorimetric method involved reduction of the product with potassium borohydrate and the later measurements of fluorescent formed lessen the NQS product after excitation at 295 nm and emission wavelength at 385 nm for the spectrofluorimetric method under optimum conditions, linear relationship with best correlation coefficient 0.9999 and the linearity was detected in between the range of 0.25-1.25 µg/mL. The mean accuracy was found to be 99.57 % to 99.66 %. The intraday and interday precision was found to be 0.14-0.16 % and 0.13-0.15% respectively. The limit of detection was found to be 0.0090µg/mL and the limit of quantification was 0.0270µg/mL. Therefore this newly recommended spectrofluorimetric method is most suitable for estimation of Memantine and observed to be validated for calculation of accuracy, precision, robustness, LOD and LOQ. So this method becomes highly congenial for analyzation of Memantine in tablet form either commercially or in dosage form with reagent of NQS. The results of analysis of this new proposed method can be compared and found to be agreeable with the results achieved through official method. Therefore spectrofluorimetric method is considered as most convenient analytical technical method for pharmaceutical estimation in quality control as well as clinical laboratories.

INTRODUCTION: Memantine Hydrochloride (MEM) [1] is the foremost in the new class of drug used for the treatment of dementia and further researchers proved its efficacy in the treatment of certain neurological disorders in addition to Parkinson's disease, spasticity, particularly moderate to severe dementia associated with alzheimer's, pervasive disorders and alcohol abuse and withdrawal. [2, 3] The chemical name of the MEM is 3, 5 - dimethyl adamantan -1 -amine and structural formula for MEM hydrochloride is shown in **Fig. 1**.

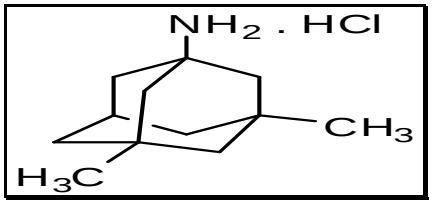

FIG. 1: CHEMICAL STRUCTURE OF MEMANTINE HYDROCHLORIDE.

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MEM empirical formula is $C_{12}H_{21}N$. HCl and a molecular weight of 215.76 which is available in the form of smooth white powder well soluble in water. It is available in tablet form consisting of 5 mg and 10 mg. Common brands of Admenta 10, sun pharmaceutical industries Ltd., Mumbai is utilized in the present research study. Any sort of official method for the determination of MEM has not yet been fourth coming. Several analytical techniques were reported for determination of MEM such as Spectrophotometric and spectrofluorimetric [4-7] high performance liquid chromatography (HPLC) coupled to MS [8, 9] and gas chromatography-mass spectrometry [10], capillary zone electrophoresis[11].

The above explained some techniques are sensitive and require high cost instruments are occasionally tedious and time taking. But the proposed spectrofluorimetric new method requires simple solvent and no need of complicated sample preparation. The new developed method of analysis was validated with reference to the guidelines of ICH Q2 (R1)[12].

A deep and through literature survey on MEM analysis reveled that NQS has not yet so far utilized as reagent to determine MEM tablets with spectrofluorimetric method. NQS has been used to determine several compounds but the reaction between the NQS as reagent and MEM in tablet dosage form has not yet been attempted. The primary aim of this research analysis study is to develop rapid, simple, accurate, sensitive and low cost alternative techniques for the determination of MEM in bulk and pharmaceutical dosage forms. The author specifically observed that for the quality control of the drugs of commercial formulations, simpler matrix are being properly used among which spectrofluorimetric method is normally used for routine analysis in quality control laboratories but literature survey speaks that estimation of MEM tablets are found to be negligible, but nobody has tried to investigate till now with NQS as reagent with spectrofluorimetric method.

So the present analysis was particularly centralized to examine NQS as derivatizing reagent in the determination of sensitive spectrofluorimetric method to estimate MEM in tablet form since it has not yet been investigated as narrated above.

MATERIALS AND METHODS:

Chemicals and reagents:

MEM hydrochloride drug was kindly supplied voluntarily by Aurobindo pharma Labs, Ltd., Hyderabad, India. 1, 2 – Naphthoquinone - 4 - sulphonate (NQS) was procured from Merck Specialties Pvt. Ltd., Mumbai, India. Potassium borohydrate (KBH_4) was procured from Merck Specialties Pvt. Ltd., Mumbai, India. Vignan pharmacy college, Vadlamudi, freely supplied triple distilled water from their own plant. All the solvents and reagents are analytical grade utilized throughout the estimation of the drug. MEM tablets of 10 mg were procured from local market.

Apparatus and Instruments:

Model SL-174 Elico Spectrofluorimeter with 1 cm quartz cells was used to obtain spectral and fluorescence measurements. ELICO LI120 pH meter was used for adjusting pH. ESSAE VIBRA AJ (0.001g), ESSAE-Teraoka Ltd weighing balance and ultrasonicator of Ultrasonic bath sonicator, PCI Ltd., Mumbai. 10 mL and 100 mL volumetric flasks, 0.5-10 µl adjustable-volume micropipet, 1mL, 5mL, 10 mL pipettes, beakers, measuring cylinders etc., were utilized in this research work.

Preparation of reagents and standard solutions:

1, 2- Napthoquinone-4-sulphonate (NQS) as derivatization reagent:

150 mg of NQS was accurately weighed and transferred in to 25 mL volumetric flask and allowed to dissolve completely in 5 mL of distilled water duly sonicated and made up to volume with distilled water and obtained 0.6 w/v solutions which was secured from exposure to light while using the solution.

Preparation of standard drug solution:

Stock solution: A quantity of 100 mg of MEM was correctly weighed and poured into 100 mL volumetric flask and kept till it is dissolved in 30 mL distilled water, sonicated for 5 minutes and the resultant solution was diluted to volume with triple distilled water to get stock solution of 1mg/mL (1000 µg/mL) concentration. This solution was utilized as a working standard solution.

Working solutions: For spectrofluorimetry, 1 mL of the stock solution was poured into the 100 mL volumetric flask and made up to the mark with

triple distilled water to get final concentration of 100µg/mL.

Preparation of sample solution:

20 MEM tablets were accurately weighed and ground into smooth powder, from it quantity of the tablet powder equivalent to 100 mg of MEM was exactly weighted and put in to 100mL of volumetric flask containing 30mL triple distilled water and sonicated continuously for 20 minutes till it was entirely dissolved and thoroughly filtered. The said filtered solution was made up to the volume with triple distilled water and obtained required concentrations to execute analysis in spectrofluorimetric method.

General recommended procedure:

Aliquots (0.025 – 0.125mL;10µg/mL) of MEM solution consisting of 0.25 to 1.25 µg/mL and from a standard solution of 10 µg/mL were transferred to a series of 10 mL of volumetric flasks and then 1mL quantity of 0.01 M NaOH and 1 mL of 0.6% w/v NQS were added. The total content of the calibrated flask and got it heated in water bath for 45 minutes at 80±5^{o}C and then kept it in ice water for about 2 minutes to come down to cooled. The total quantity in the calibration flask was transferred in to a separating funnel and then to get it extracted with 2 portions of 5 mL quantity of chloroform.

The said combination of chloroformic extracts was allowed to evaporate under stream of air. The remaining portion was mixed with 2 mL methanol and then quantitatively transferred into 10 mL calibrated flask, Again 1mL of potassium borohydrate solution with concentration of 0.03% in methanol was added and kept at room temperature of 25 ±5^{o}C for 5 minutes to get reaction. The solution obtained due to reaction was filled up to the mark with 0.025 M ethanolic HCl and the resultant intensity of fluorescence of the solution was measured at 385 nm soon after excitation at 295 nm against reagent blanks treating in the same way. The amount of MEM present in the sample solution was calculated from the calibration curve.

Setting of wavelength:

For the wavelength detection the working standard solution of MEM hydrochloride was scanned wavelengths ranging from 200-600 nm in spectrofluorimeter and got 295 nm as excitation wavelength and 385 nm as the emission wavelength and the results so obtained are graphically shown in **Fig. 2 and 3.**

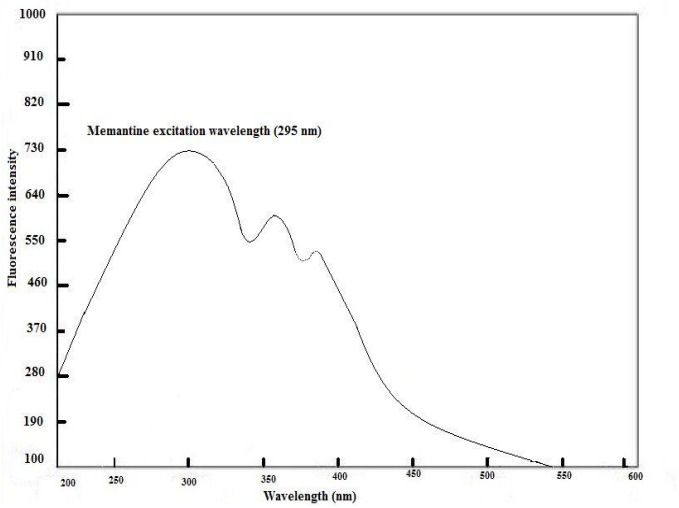


FIG. 2: EXCITATION SPECTRUM OF MEM WITH NQS SYSTEM.

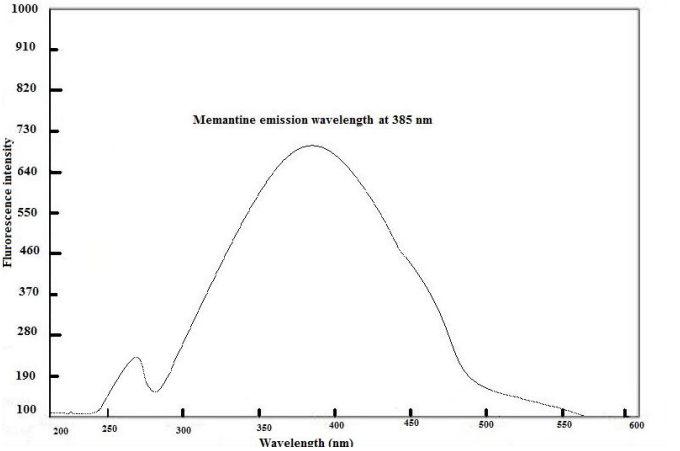


FIG. 3: EMISSION SPECTRUM OF MEM WITH NQS SYSTEM.

Optimization and method development:

A reduction action on MEM-NQS product is direly needed to estimate the spectrofluorimetric method. The reduced NQS reagent works as fluorescent in the maximum identical excitation as well as emission product of MEM - NQS. Therefore it is necessary before taking up the reduction process a selected extraction step for the MEM-NQS product out of left out reagent of NQS. Based on the reported efficiency potassium borohydrate was selected as reducing reagent for derivatives of NQS. By changing the concentrations ranging 0.001- 0.01% w/v the reaction was conducted and then took up the investigation to detect the effect of

potassium borohydrate being a reducing agent. To detect fluorescence intensity was got in the final solution of 1mL of 0.03 w/v at the concentration of 0.003% but it was noticed that the concentrations beyond 0.003% yield no effect on the fluorescence intensity of reaction product of MEM with NQS **(Fig.4).**

The effect of pH on the relative fluorescence intensity (RFI) of the reduced reaction product of MEM-NQS results disclosed that maximum fluorescence intensity was attained at pH 2.0 **(Fig. 5)** after reaction mixture was diluted with 0.025 M ethanolic HCl solution. The results of optical characteristics and regression data of proposed method is shown in **Table 1.**

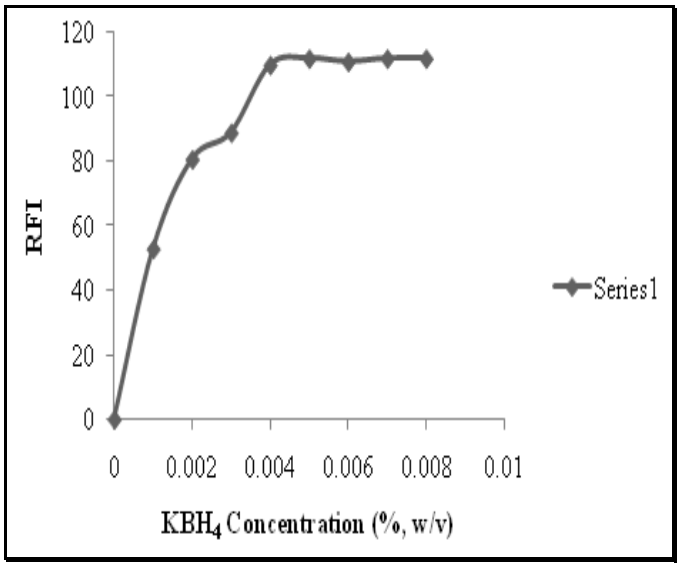


FIG. 4: EFFECT OF KBH_4 CONCENTRATION ON THE RFI

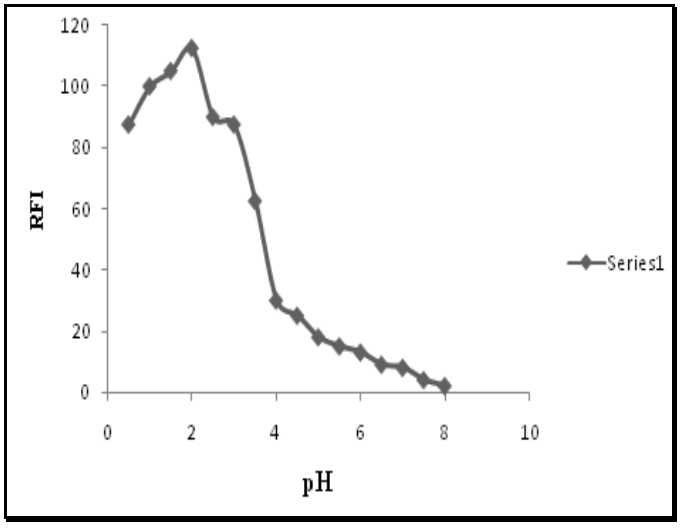


FIG. 5: EFFECT OF PH ON THE RFI

TABLE 1: OPTICAL CHARACTERISTICS AND REGRESSION DATA FOR PROPOSED SPECTROFLUORIMETRIC METHOD

Parameter	Results
λ_{ex} (nm)	295
λ_{em} (nm)	385
Linearity range (µg/mL)	0.25 -1.25
Regression equation (Y= a + bc)	Y = 536.91x – 0.2381
Intercept(a)	-0.2381
Slope (b)	536.91
Standard deviation of intercept (S_a)	1.4663264
Standard deviation of slope (s_b)	1.9372468
Standard error of estimation (S_e)	2.0260212
Correlation coefficient (r^2)	0.9999
% Relative standard deviation*	0.16%
Limit of detection (µg/mL)	0.0090
Limit of quantitation (µg/mL)	0.0270
% Range of Error (Confidence limits)*	
0.05 level	0.5182
0.01 level	0.6810

** n =6. Average of six determinations.

Method validation:

Linearity:

Various aliquots were prepared from working solutions of MEM - NQS (10 µg/mL) ranging from 0.025, 0.05, 0.075, 0.1, 0.125 mL were transferred in to a series of 10 mL volumetric flasks and the volume was brought up with triple distilled water to attain the 5 different concentrations of the drug ranging from 0.25-1.25 µg/mL of solution. The fluorescence intensity was measured at 385 nm over the concentration range of 0.25-1.25 µg/mL and calibration curve was formed by plotting Concentration of MEM - NQS (ppm) on x-axis and resultant fluorescence intensity on y-axis and regression equation was calculated. The calibration curve of Memantine drug is shown in **Fig. 6.** The Calibration data of standard Memantine is represented in **Table 2.**

TABLE 2: CALIBRATION DATA OF STANDARD MEMANTINE

Concentration (µg/mL)	Fluorescence intensity
0	0
0.25	134
0.5	267
0.75	405
1	534
1.25	672

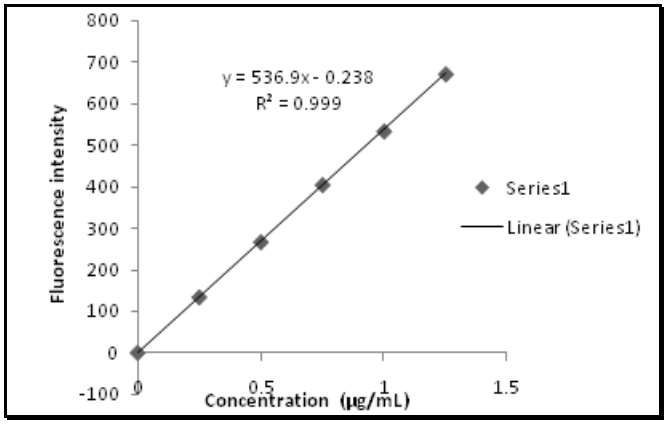


FIG. 6: CALIBRATION CURVE OF MEMANTINE

Accuracy:

The accuracy was determined by calculating the recovery of Memantine hydrochloride by standard addition method. To a fixed amount of Memantine (1 µg/mL) of pre-analyzed sample of Memantine hydrochloride, increasing amount of Memantine hydrochloride was added at all levels of calibration curve and the amount of MEM was calculated at each level and the results obtained are shown in **Table 3.**

TABLE 3: ACCURACY DATA OF MEMANTINE

Initial conc. (µg/mL) (A)	Quantity of Std.added (µg/mL) (B)	Total amount (A+B)	Total quantity found mean[a] ± SD[b]	% Recovery[$]±SD[b]
1µg/mL	2 µg/mL	3 µg/mL	2.99±0.51	99.66±0.62
1µg/mL	4 µg/mL	5 µg/mL	4.98±0.23	99.6±0.42
1µg/mL	6 µg/mL	7 µg/mL	6.97±0.34	99.57±0.45

[$] = Three times repetition were done, [a]Average of five determinations, [b]SD = Standard deviation.

Precision:

Repeatability (Precision on replication):

From working solution (100µg/mL) aliquot of 0.1 mL was transferred to the 10 mL volumetric flask and made up to mark with triple distilled water (1µg/mL). The fluorescence intensity of this solution was measured at 385nm. The fluorescence intensity of the same solution was measured 5 times and % CV was calculated.

Intraday and Interday precision:

Intraday precision was decided by analyzing Memantine hydrochloride (0.25, 0.75 and 1.25 µg/mL) for 3 times in the same day and % CV was calculated. Interday precision was determined by analyzing Memantine hydrochloride (0.25, 0.75 and 1.25 µg/mL) daily for 5days and % CV was calculated. The results of intra and inter day precision of MEM by spectrofluorimetry is presented bellow **(Table 4).**

TABLE 4: INTRA DAY AND INTERDAY PRECISION OF MEMANTINE

Concentration(µg/mL)	Intraday precision[$]		Interday precision[$]	
	Flurescence intensity Mean[a]± SD[b] (n=5)	%CV[c]	Flurescence intensity Mean[a]± SD[b] (n=5)	%CV[c]
0.25 µg/mL	134±0.125	0.15	135±0.242	0.13
0.75 µg/mL	405±0.312	0.16	405±0.258	0.15
1.25 µg/mL	672±0.341	0.14	673±0.311	0.13

[$] = Three times repetition were done, [a]Average of five determinations, [b]SD = Standard deviation. [c]%CV = Coefficient of variation.

LOD: Based on the standard deviation of the response and the slope the limit of detection may be expressed as LOD=3.3*s/S. Where s= the standard deviation of the response. S= the slope of the calibration curve.

LOQ: Based on the standard deviation of the response and the slope The quantification limits may be expressed as LOD = 10s/S where s= the standard deviation of the response. S= the slope of the calibration curve.

Application to pharmaceutical preparation:

It is evident from the above mentioned results that the proposed methods gave satisfactory results with Memantine in bulk. Thus its tablets were subjected

to the analysis of their contents form the active ingredient by proposed method. To determine the content of MEM in tablet (label claim 10mg /tablet) the contents of 20 tablets were weighed and their mean weight determined and finely powdered. An equivalent weight of the tablet content was transferred into a 100 mL volumetric flask containing 60 mL of triple distilled water, sonicated for 20 minutes and filtered through whatman filter paper. The procedure described under general recommended procedure previously discussed was followed. Concentration of sample solution was found from calibration curve of Memantine hydrochloride. The determination of MEM in tablet dosage form by spectrofluorimetry is shown in **Table 5.**

TABLE 5: ESTIMATION OF MEMANTINE HYDROCHLORIDE IN TABLET BY SPECTROFLUORIMETRY

Tablet formulation	Labeled claim (10 mg/tablet)	Amount found[a] (mg/tablet)	% recovery* ±SD[b]
Admanta 10	10 mg	9.98 mg	99.8±0.15

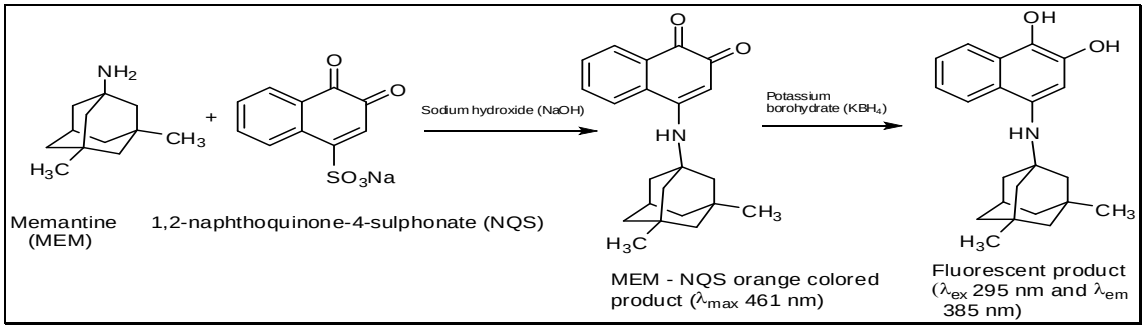
* = 3 times repetitions were done, [a]Average of 5 determination, [b] = Standard deviation

RESULTS AND DISCUSSION:

This method concerned with determination of Memantine by measuring the flurescence intensity at emission wavelength at 385 nm. This method was found to be linear in the range of 0.25-1.25 µg/mL having correlation coefficient r^2 =0.9999 **(Figure 6, Table 1)**. The mean accuracy was found to be 99.57% to 99.66% **(Table 3)**. The intraday and interday precision was found to be 0.14-0.16% and 0.13-0.15% respectively **(Table 4)**. The limit of detection was found to be 0.0090 µg/mL and the limit of quantification was 0.0270µg/mL. After words the validity of the method was again checked by adopting the standard addition method. The results of the proposed new invented method were specifically showed that it is valid and can be applied for the estimation of MEM in pharmaceutical preparations.

Owing to non presence of any amount of chromophoric group in molecule of MEM it could not absorb in the ultra violet visible region beyond 200 nm as well as no native fluorescence as a result of which in the current study derivatization of MEM was tried to develop the spectrofluorimetric method to determine MEM For primary and secondary amines, NQS was utilized as reagent for chromogenic and fluorogenic purpose. Even then the resultant reaction with MEM as yet to be examined in detail. That is why the current study was concentrated to investigate NQS as a chief derivating reagent while developing spectrofluorimetric method for estimation of MEM in tablet form. Therefore the primery experiments for getting reaction between MEM-NQS make known clearly that the product of MEM-NQS obtained was attained orange colour showing optimum absorption at 461 nm which was not soluble in water but soluble in organic solvents only.

The reduced MEM-NQS derivative was detected to be fluorescent and showed excitation 295 nm and emission wavelength at 385 nm and the present study was carried out at the corresponding wavelength. The Figure furnished bellow showed the absorption and excitation as well as emission as spectra corresponding with product reaction. **Scheme 1** shows the reaction pathway between MEM and NQS.



SCHEME 1: THE REACTION PATHWAY OF MEMANTINE WITH NQS

CONCLUSION: After going through the facts of relevant literature on this subject the author observed that no analyst hitherto analyze MEM drug in tablet form with NQS as reagent with spectrofluorimetric method. Therefore the invented simple and sensitive spectrofluorimetric method successfully carried out the analyzation of MEM as stated above and the results pertaining to the statistical analysis conformed that the present developed method possesses good precision and accuracy without interference of normal additives existed in the pharmaceutical preparations. The current study illustrated the utility of NQS as reagent for the estimation of MES drug with spectrofluorimetric method in bulk and pharmaceutical dosage form and found that this developed method is better than previously reported methods with regard to its selectivity and sensitivity features.

The linearity range of the proposed spectrofluorimetric method is less than previously reported methods on MEM. More over all the reagents connected with this analytical method are less cost having shelf life and are easily procured and preserved in any analytical laboratory. The statistical results of the analysis of the tablets by this method were reproducible, reliable and were good agreement with labeled claim of the drug. There is no interference of the present excipients in the tablets. Finally it is concluded that this method can be applied for the routine determination of MEM in pharmaceutical formulation.

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