A SENSITIVE SEQUENTIAL INJECTION ANALYSIS (SIA) DETERMINATION OF MEMANTINE HYDROCHLORIDE USING LUMINOL-HYDROGEN PEROXIDE INDUCED CHEMILUMINESCENCE DETECTION

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ABSTRACT

A fully automated sequential injection analysis (SIA) method for chemiluminescence (CL) determination of Al-zheimer's drug memantine hydrochloride was developed and presented. The employing of SIA injection analysis facilitates fluidic handling and lowering the consumption of sample and reagents. The basic CL reaction was based on the emission of CL radiation due to the reaction of luminol with hydrogen peroxide in basic medium. The CL emission has been monitored using FIAlab system. The optimum conditions and characterizations were optimized using a computer-aided simplex method. Under the optimal conditions the linear calibration graph was obtained within the range of 0.0001-1.0 μg mL^{-1}, (r=0.9996) with detection limit of $3.3x10^{-5}$ μg mL^{-1} along with relative standard deviation 1.8 % (n). The flow rates were 120 μL s^{-1}, for the aspiration into the holding coil and 80 μL s^{-1} for detection. The sample frequency throughput was 103 h^{-1}. The proposed CL method was successfully applied for determination of the tested drug in pure form and its pharmaceutical formulations. The interference of some common additive compounds such as glucose, lactose, starch, talc and magnesium stearate was investigated. No interference was recorded. The obtained SIA results were statistically compared with those obtained from a reported method and did not show any significant difference.

Keywords: Sequential injection analysis; Chemiluminescence determination; Memantine hydrochloride; Pharmaceutical formulations

1. INTRODUCTION

Memantine hydrochloride (Figure 1) chemically known as 1-amino-3,5 dimethyladamantane hydrochloride. Memantine is used to treat the symptoms of Alzheimer's disease. It is in a class of medications called *N*-Methyl-*D*-aspartate receptor (NMDA) receptor antagonists. This medication works by decreasing abnormal excitement in the brain. Memantine can help people with Alzheimer's disease to think more clearly and perform daily activities more easily. Memantine may also be prescribed to treat other medical conditions [1]. Because of its important and intensive medicinal uses several methods have been proposed for its determination in pharmaceutical formulations and biological fluids. These mainly include high performance liquid chromatography [2-4], liquid chromatography coupled with mass spectrometry [5, 6], gas chromatography [7, 8], spectrophotometry [9-11] and spectrofluorimetry [11].

The use of SIA-injection chemiluminescence seems to be quite important during recent years. This can be attributed to the fair sensitivity of such analytical technique that can be employed in the determination of different compounds in various fields.

In the present study a simple, accurate and reliable SIA-injection CL system was adopted for the determination of memantine hydrochloride in pure form and its dosage forms. The utilization of luminol-hydrogen peroxide system together with SIA injection allows minimizing the consumption of reagents and samples. Method validation for the proposed SIA-CL method for the determination of memantine hydrochloride was carried out according to ICH guidelines [12].

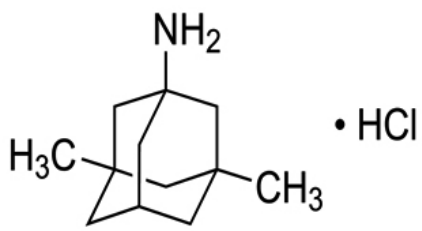

Figure 1: Chemical structure of memantine hydrochloride

2. EXPERIMENTAL

2.1. Instrumentation

SIA system (FIAlab-3500 instrument, USA) comprised of a CAVRO XL 3000 syringe pump volume 2.5 mL (Cavro Scientific Instrument Int., USA) and Vici Valco Cheminer RT® 125-0718 eight-port manifolds. Fluorimetric/ Chemiluminescence detector (UIV lamp switched off) equipped with a lab-made CL module with spiral geometry; the photomultiplier tube voltage was 320V. Autosampler model ALM 3200. The SIA system involved a holding coil (length 70 cm, i.d. 0.8 mm, PTFE tubing volume 1.2 mL). The same tubing was spirally coiled on a 52 mm×52 mm Perspex plate, which substituted the secondary filter in the fluorimeter; this CL module had a central inlet, peripheral outlet and the diameter of the spiral was 24 mm. The SIA unit was PC controlled and data acquisition was performed with (FIAlab for windows version 5.9.321) software. The solution stability monitoring and UV spectrophotometry was performed on an UV-Visible Spectrophotometer Ultrospec (model 2100 pro).

2.2. Reagents

Distilled water and analytical grade reagents were used throughout the work. Pure grade memantine hydrochloride was kindly supplied from Adwia Co. Egypt. $5.0x10^{-4}$ mol L^{-1} luminol (Sigma Chemical Co.) stock solution was prepared in 100 mL of $1.0x10^{-2}$ mol L^{-1} sodium hydroxide (WINLAB). Hydrogen peroxide 30% (WINLAB) was used to prepare $1.0x10^{-3}$ mol L^{-1} by appreciated dilution using distilled water. Cationic surfactants such as cetylprydenium chloride (CPCl), cetyltrimethylammnium bromide (CTAB), non-ionic surfactants such as glycerol, tween-80 and triton-X 100 and anionic surfactants such as sodium lurayl sulphate (SDS) and 1,2-naphoqinon-4-sulphonate (NQS) were purchased from (Sigma Aldrich, Germany). The pharmaceutical preparation (Ravemantine®10 mg/tablet, EVA, Pharm. Egypt) was purchased from local drug stores.

2.3. Standard drug solution

A stock standard memantine hydrochloride solution 100 μg mL^{-1} was prepared by dissolving 10 mg of pure drug in 100 mL distilled water. Working solutions were prepared daily by appropriate dilution. The employed working solutions were in the range of 0.0001- 1.0 μg mL^{-1}.

2.4. Manifold and procedures

The following procedure was carried out for the determination of memantine hydrochloride. All experiments were computer controlled to ensure timing of syringe pump and valve movements. The schematic diagram of SIA-CL system as shown in Figure 2 was employed for automated aspiration of the appropriate defined volumes of standard and test solutions of analyte and reagents. Prim-port program was used first to fill in the lines connected with the test solution and reagents. The sequence of the aspirated sample and reagents was automatically controlled. Mixture of 80 μL luminol $5.0x10^{-4}$ mol L^{-1}, 60 μL sample solution and 80 μL hydrogen peroxide $1.0x10^{-3}$ mol L^{-1} was aspirated into the holding coil through the eight-way injection valve at a flow rate 120 μL s^{-1} and then the mixed solution was flushed continuously into the flowthrough cell located in front of detection cell of the photomultiplier tube (PMT). The resulting peak heights were calculated automatically by FIAlab® supported software version 9.5.321. The luminescing zone was dispensing into detector using pre-programmed measuring cycles. Each measuring cycle was carried out in triplicate and the mean peak heights were used in the evaluation of the experiments. All measurements were carried out at ambient temperature

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25±1˚C. During the SIA-CL detection of memantine hydrochloride about 1000 µL distilled water was used as carrier for delivering sample and reagents zones into the flow cell for measuring the CL signal. The obtained data was stored in the PC for subsequent processing.

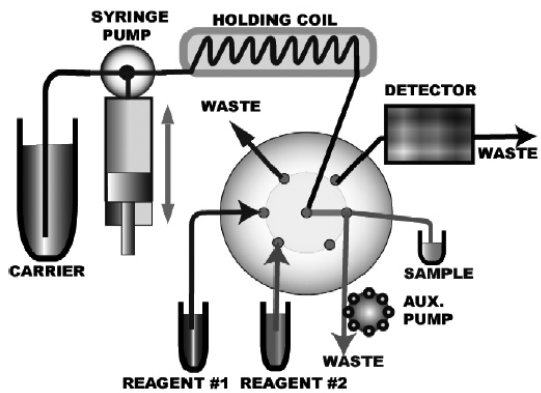

Figure 2: Schematic diagram of SIA injection system for chemiluminescence determination of memantine hydrochloride; carrier stream (water); reagent 1 (luminol $5.0x10^{-4}$ mol L^{-1}); reagent 2 (hydrogen peroxide $1.0x10^{-3}$ mol L^{-1}); sample (memantine hydrochloride 0.1 µg mL^{-1})

2.5. Calibration

Under the optimum conditions the calibration curve for determination of memantine hydrochloride was obtained. The graph related the CL intensity vs. the concentration of tested drug solutions was plotted at 12 experimental points. The mean peak heights were obtained after triplicate sample aspiration. Conventional linear regression was utilized for fitting the curve.

2.6. Analytical applications

2.6.1. Determination of memantine hydrochloride in ravemantine® tablets

To determine memantine hydrochloride in its dosage form (Ravemantine® 10 mg/ tablet) twenty tablets were finely powdered and weighed. An amount of powder equivalent to 10 mg memantine hydrochloride was dissolved in distilled water and then sonicated for 10 min. The sonicated solution was filtered using membrane filter (pore size 5.0 µm) and complete to 100-mL. The working solutions were prepared by serial dilutions in the range of 0.001-1.0 µg mL^{-1}. The proposed SIA-CL method was employed to determine the investigated drug in each concentration. The mean % recoveries were calculated using calibration graph.

2.6.2. Content uniformity assay of tablets

The content uniformity assay of memantine hydrochloride (Ravemantine® 10 mg /tablet) was carried out using the proposed SIA-CL detection. Ten individual tablets were dissolved separately in 100 mL distilled water. The prepared solutions were sonicated for 10 min then filtered through membrane filter (pore size 0.5µm). 1.0 mL of the previously filtered solution was transferred into a conical flask and complete to 100 mL with distilled water to obtain a test solution containing 1.0 µg mL^{-1} of memantine hydrochloride. The SIA-CL method was employed to determine the content uniformity assay of the tablets using the calibration graph.

3. RESULTS AND DISCUSSION

3.1. Optimization studies

3.1.1. Selection of hydrogen peroxide as oxidizing agent

To select the suitable oxidizing agent various oxidants were carefully investigated including potassium ferricyanide, potassium permanganate, potassium periodate, hydrogen peroxide and Ce (IV). It was seen that no CL signal was recorded on using all these oxidizing agents. While, on using luminol with hydrogen peroxide exhibits a CL signal in the presence of alkaline medium. Therefore, the proposed procedure was based on the enhancement effect of memantine hydrochloride on the luminol-hydrogen peroxide- CL signal and the effect of luminol and hydrogen peroxide concentrations was further investigated and optimized.

3.1.2. Effect of luminol and hydrogen peroxide concentrations

The influence of luminol and hydrogen peroxide concentrations on the CL signal was investigated using $1.0x10^{-5}$-$1.x10^{-1}$ mol L^{-1} for both reagents. As shown in Figure 3, it was found that the CL intensity showed significant increase at $5.0x10^{-4}$ and $1.0x10^{-3}$ mol L^{-1} for luminol and hydrogen peroxide, respectively. Therefore, these concentrations are chosen for the subsequent experimental analysis.

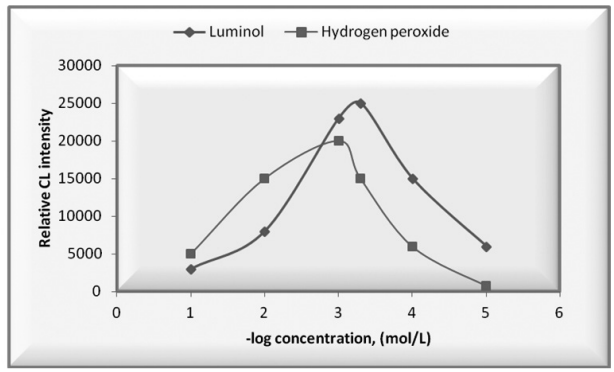

Figure 3: Effect of luminol and hydrogen peroxide concentrations, for luminol concentration, (memantine hydrochloride 0.1 µg mL^{-1} and hydrogen peroxide $1x10^{-3}$ mol L^{-1}) and for hydrogen peroxide concentration (memantine hydrochloride 0.1 µg mL^{-1} and luminol $5.0x10^{-4}$ mol L^{-1})

3.1.3. Optimization of alkaline medium

In order to select and investigate the suitable alkaline medium as a solvent for luminol, three kinds of alkaline media including ammonium hydroxide, sodium bicarbonate and sodium hydroxide in the range $1.0x10^{-3}$-$1.0x10^{-1}$ mol L^{-1} were examined. As shown in Figure 4, it was found that the use of $1.0x10^{-2}$ mol L^{-1} sodium hydroxide gave a sharp CL signal while in the cases of ammonium hydroxide and sodium bicarbonate a significant decrease in CL signal was obtained. Therefore, $1.0x10^{-2}$ mol L^{-1} sodium hydroxide was used in the proposed method as a solvent for luminol.

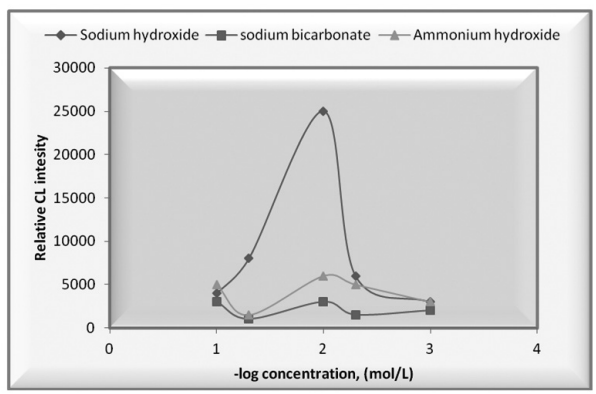

Figure 4: Effect of sodium hydroxide and sodium bicarbonate concentration on CL intensity system (memantine hydrochloride 0.1 µg mL^{-1}, hydrogen peroxide $1x10^{-3}$ mol L^{-1} and luminol $5.0x10^{-4}$ mol L^{-1})

3.1.4. Optimization of aspirated volumes of sample and reagents

The aspirated volume of sample and reagents was considered as very critical parameter which should be governed and carefully optimized. To optimize the aspirated volume of sample and reagents computer-aided simplex method was used. The proposed method was carried out by varying the volume of sample and CL reagents solutions. As shown in Figure 5a, it was clear that for sample, the CL intensity increased with increasing sample zone volume up to 60 µL and then kept unchangable. This may be attributed to adjacent sample-reagent zones and disperse to each other to form the CL reaction. Moreover for CL reagents the optimum aspirated volume was 80 µL. The time was extended to 35 s for complete flushing through the holding cell with carrier in between analysis cycles. Also the influence effect of the flow rate on CL intensity was investigated in the range of 10-150 µL s^{-1}. It was noticed that the CL intensity was increased with the increase of flow rates. The optimum flow rate was found to be 120 µL s^{-1} and was used for further studies (Figure 5b).

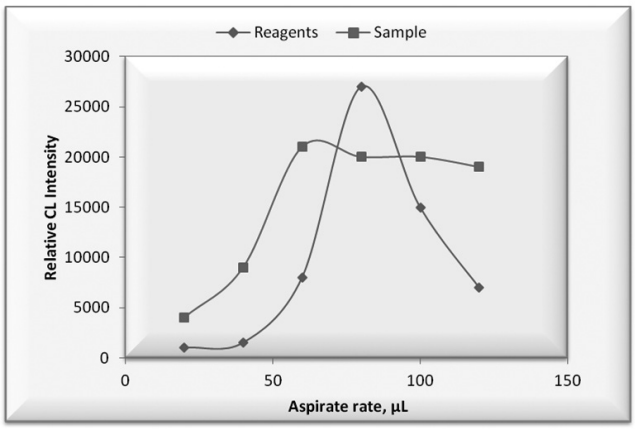

Figure 5a: The influence of aspirated volumes of memantine hydrochloride and reagents (10-150 µL) on the relative CL intensity. Conditions: memantine hydrochloride 0.1 µg mL^{-1}; hydrogen peroxide $1.0x10^{-3}$mol L^{-1} and luminol $5.0x10^{-4}$ mol L^{-1}

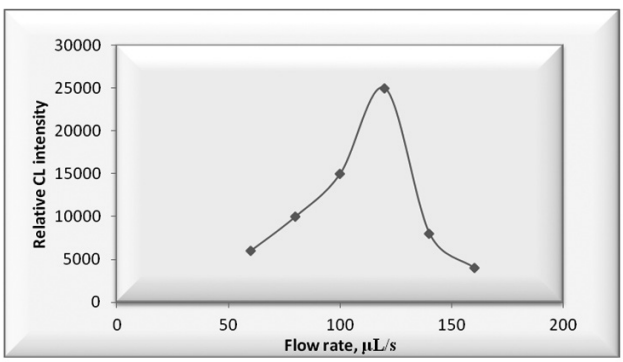

Figure 5b: The influence of flow rate on the relative CL intensity. Conditions; memantine hydrochloride 0.1 µg mL^{-1}; 80 µL hydrogen peroxide $1.0x10^{-3}$ mol L^{-1}; 80 µL luminol $1.0x10^{-4}$ mol L^{-1} and pH 10

3.2. SIA control program

In order to perform all calibration measurements and experimental analysis of memantine hydrochloride, SIA control program was utilized. The utilized program also used to carry out the assay of memantine hydrochloride in its pharmaceutical dosage forms. The typical sequence of particular steps of program is presented in Table 1. It was clear that the single cycle takes about 35 s therefore, the sample throughput of 103 h^{-1} can be recorded.

3.3. Characterizations

SIA-CL luminol-hydrogen peroxide system was applied for the determination of memantine hydrochloride. The presented results in Table 2, clarified that the measurable linear concentration range was 0.0001-1.0 µg mL^{-1}, (r = 0.9996) with lower limit of detection of $3.3x10^{-5}$ µg mL^{-1} and quantification limit of $1.0x10^{-4}$ µg mL^{-1}. The regression parameters were calculated from the calibration graph, the reproducibility of the proposed method was tested using twelve drug test solutions and the relative standard deviations were less than 5% indicating that the proposed method was suitable for routine analysis of the investigated drug.

3.4. Effect of foreign substances

In order to determine memantine hydrochloride in its pharmaceutical dosage form, the interference of some common ions and excipients was investigated. To evaluate the interferences a test solution of 0.1 µg mL^{-1} of memantine hydrochloride was treated with appropriate foreign substance to contain ≈ 1.0 mg mL^{-1}. The mean peak heights were compared with those obtained with pure 0.1 µg mL^{-1} analyte solution. The results of tolerable concentration level for interference at 5% level were listed in Table 3. It was clear that no interferences have been seen in the determination of memantine hydrochloride in its pharmaceutical dosage forms.

3.5. Effect of surfactants

To study the effect of surfactants on SIA-CL luminol-hydrogen peroxide system for determination of memantine hydrochloride different kinds of surfactants were investigated. Cationic surfactants such as cetylprydenium chloride (CPCl), cetyltrimethylammonium bromide (CTAB), non-ionic surfactants such as glycerol, tween-80 and triton-X 100 and anionic surfactants such as sodium dodecyl sulphate (SDS) were tested. 0.1 µg mL^{-1} of the investigated drug memantine hydrochloride was treated with appropriate amount of surfactant ≈ 1.0 mg and the SIA-CL method was proposed for detection. The CL signal was recorded for each surfactant and the peak heights of the signals were compared with those of pure memantine hydrochloride 0.1 µg mL^{-1}. As cleared in Figure 6, no enhancement effect was obtained.

Table 1: The control program of SIA-chemiluminescence detection of memantine hydrochloride with luminol and hydrogen peroxide system

Device	Command	Parameter	Action
Loop Start (♯) 1			
Next sample			
Peristaltic pump	Counter clockwise	% 50	
	Delay (s)	35	
	Peristaltic pump off		
Detector	ON		
Syringe pump	Valve position IN		
Syringe pump	Set flow rate (µL s^{-1})	100	
Syringe pump	Aspirate (µL)	1500	Pump filled with carrier
Syringe pump	Delay until done		
Multiposition valve	Set valve position	3	
Syringe pump	Set flow rate (µL s^{-1})	120	
Syringe pump	Aspirate (µL)	80	Reagent A (Luminol $1.0x10^{-3}$ mol L^{-1})
Multiposition valve	Set valve position	5	
Syringe pump	Set flow rate (µL s^{-1})	120	
Syringe pump	Aspirate (µL)	60	Sample (Memantine Hydrochloride)
Syringe pump	Delay until done		
Multiposition valve	Set valve position	4	
Syringe pump	Set flow rate (µL s^{-1})	120	
Syringe pump	Aspirate (µL)	80	Reagent B (Hydrogen peroxide$1.0x10^{-2}$ mol L^{-1})
Syringe pump	Delay until done		
Multiposition valve	Detector	7	
Syringe pump	Set flow rate (µL s^{-1})	120	
PMT	Start scan		
Syringe pump	Empty		
Syringe pump	Delay until done		
PMT	Stop scans		
Refresh plat			
Loop end			

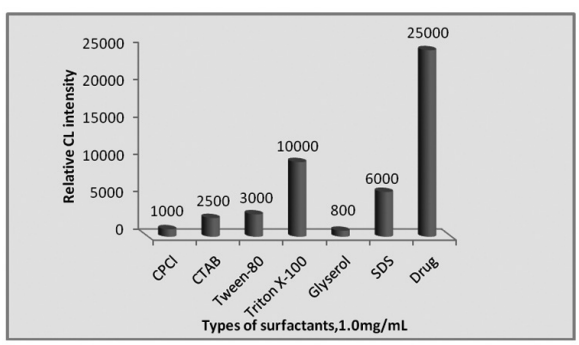

Figure 6: Effect of surfactants (1.0 mg mL^{-1}) on SIA-CL system (memantine hydrochloride 0.1 µg mL^{-1}, hydrogen peroxide $1x10^{-3}$ mol L^{-1} and luminol $5.0x10^{-4}$ mol L^{-1})

3.6. Analytical applications

The proposed SIA-injection CL was used for the determination of memantine hydrochloride in its dosage forms. The obtained results were

presented in Table 4 and statistically compared by Student's t-test [13] with those obtained from the reported spectrophotometric method [9]. The results did not reveal any significant difference between them. The content uniformity assay for memantine hydrochloride tablets was investigated and the results were presented as the mean % recoveries and standard deviation (99.24 ± 1.6). To improve further accuracy and precision for the proposed method the results in terms of linear concentration range and lower limit of detection obtained from the determination of the proposed method were compared with those recorded in previously reported methods as summarized in Table 5.

3.7. Method validation

Method validation was carried out with respect to linearity, lower limit of detection, quantification limit, accuracy, precision, and robustness according to ICH guidelines [12].

3.7.1. Linearity

The proposed SIA-CL method for determination of memantine hydrochloride using luminol-hydrogen peroxide system was successfully applied for evaluation of the linear concentration range. Visual inspection of a plot of signals as a function of memantine hydrochloride concentrations was used. Twelve standard solutions in the concentration range of 0.0001-10.0 µg mL^{-1} were subjected to SIA-CL detection. The regression line was calculated using least square statistical method. The results obtained clarified that the proposed SIA-CL method exhibits a linear concentration range at 0.0001-1.0 µg mL^{-1}.

Table 2: Tolerable concentration level of interferents to 0.1 µg mL^{-1} memantine hydrochloride

Interferents	Tolerable level µg mL^{-1}
Na^+, K^+, Mg^{2+}, Cl^-,NO_3^-, NH_4^+ and SO_4^{2-}	>100
Glucose, sucrose, lactose, Talc, Starch	75
Uric acid, magnesium stearate, citric acid, oxalic acid	5
Adrenaline, dopamine, cystine, histamine, tyrosine, glucosamine	25
Cd^{2+}, Co^{2+}, Fe^{2+}, Fe^{3+}, Mn^{2+}, Ni^{2+}, and Cu^{2+}	0.5

Table 3: Performance data obtained from the determination of memantine hydrochloride using luminol and hydrogen peroxide system

Analytical characteristics	Obtained results
Linear range µg mL^{-1}	0.0001-1.0
Detection limit µg mL^{-1}	$3.3x10^{-5}$
Quantification limit µg mL^{-1}	$1.0x10^{-4}$
Intercept on the ordinate	2095.2
Slope	100.5
%RSD for 0.1 µg mL^{-1} (n=12)	1.8 %
Correlation coefficient, r	0.9996

3.7.2. Lower limit of detection LOD

In order to evaluate the lower limit of detection of the proposed SIA-CL methodfor determination of memantine hydrochloride signal-to-noise ratio was performed. The lower limit of detection was calculated by S/N=3 as the concentration of memantine hydrochloride CL signal exceeds three times that of blank signal.The recorded signals showed lower limit of detection of $3.3x10^{-5}$ µg mL^{-1}.

3.7.3. Quantification limit

The proposed SIA-CL method for determination of memantine hydrochloride was employed for the determination of quantification limit of the investigated drug. Signal-to-noise ratio was performed for such evaluation. The obtained result was $1.0x10^{-4}$ µg mL^{-1} using 10:1 signal-to noise ratio.

3.7.4. Accuracy

The accuracy of the proposed SIA-CL method was carried out by investigating the tested drug in its placebo sample magnesium stearate using standard addition method.

Table 4: Determination of memantine hydrochloride using SIA-injection CL detection in pure form and dosage forms

Sample	Taken µg mL^{-1}	Found	% Recovery	
Pure solution	1.010^{-4}	$9.99x10^{-5}$	99.90	Reported method [9]
	5.010^{-4}	$4.99x10^{-4}$	99.80	
	1.010^{-3}	$9.99x10^{-4}$	99.85	
	5.010^{-3}	$4.92x10^{-3}$	98.40	
	1.010^{-2}	$9.91x10^{-3}$	99.06	
	1.010^{-1}	$1.00x10^{-1}$	100.00	
	1.0	0.9996	99.96	
% Mean ± SD	99.57±0.61			99.84±0.83
n	7			6
Variance	0.37			0.64
%SE	0.23			0.32
%RSD	0.61			0.83
t-test	0.685(2.201)*			
F-test	1.73(4.39)*			
Ravemantine® 10 mg/ tablets	1.010^{-3}	9.98710^{-4}	99.87	99.84±0.83
	5.010^{-3}	4.99810^{-3}	99.96	6
	1.010^{-2}	1.010^{-2}	100.00	0.64
	5.010^{-2}	4.93610^{-2}	98.72	0.32
	1.010^{-1}	9.97810^{-2}	99.78	0.83
	$5.0x10^{-1}$	4.95710^{-1}	99.14	
	1.0	1.0	100.00	
% Mean ± SD	99.68±0.48			
n	7			
Variance	0.23			
%SE	0.18			
%RSD	0.48			
t-test	0.435(2.201)*			
F-test	2.78(4.39)*			

Table 5: Comparative analytical results relevant to the terms of linear concentration range and detection limit between the proposed SIA-CL injection and other reported methods

Method	Linear range (µg mL^{-1})	LOD (µg mL^{-1})	Reference
Proposed SIA-CL injection	0.0001-0.1	$3.3x10^{-5}$	-
HPLC-UV detection	70-130	-	[2]
HPLC-Charged aerosol detector	0.4-0.6	-	[3]
HPLC-Fluorescence detection	0.002-0.08	-	[4]
GC-Flame ionization detection	0.971-1.789	0.437	[8]
Spectrophotometry	4-12	-	[9]
Spectrophotometry and spectrofluorimetry	5-70 and 0.02-0.2	0.05	[10]

The obtained results were calculated in terms of mean percentage recoveries values. The calculated % recovery was 99.15±0.6.

3.7.5. Precision

The precision of the proposed SIA-CL method was tested in the terms of intra-day and inter-day. The studies were carried out by repeating the determination to nine replicates. The calculated % RSD values were 1.4%, for determination of memantine hydrochloride in (Ravemantine®10 mg/tablet). The above % RSD value is less than 2% indicating good precision.

3.7.6. Robustness

The robustness of SIA-CL method for determination of memantine hydrochloride was investigated by introducing small changes on method parameters. In the proposed study the robustness of the method was carried out by changing the pH of the test solution 10 ± 1 using 1.0 mol L^{-1} sodium hydroxide or hydrochloric acid and the percentage recoveries were calculated.

The calculated % recovery for the proposed method was 99.26±0.9. The obtained results were closely in agreement with those obtained from standard drug solutions.

CONCLUSION

A sequential injection CL method for the quantitative determination of memantine hydrochloride was successfully developed. The method is based on the reaction of luminol with hydrogen peroxide in alkaline medium of pH 10. The CL intensity is substantially enhanced by memantine hydrochloride. The SIA injection system showed good stability and satisfactory detection limit. Also, the proposed method offers advantages of simplicity, reproducibility, short response time and high sensitivity for determination of memantine hydrochloride whether being in pure form and its dosage forms. The employment of automated SIA-CL technique was significantly improved the precision of CL detection and control the consumption of samples and reagents. No interference was found from the additive compound to memantine hydrochloride dosage forms.

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