World Journal of Pharmaceutical research

Volume 2, Issue 6, 1958-1970. Research Article ISSN 2277 – 7105

UTILITY OF OXIDATION-REDUCTION REACTION FOR THE SPECTROPHOTOMETRIC DETERMINATION OF ANTIVIRAL AND ANTI-PARKINSONIAN DRUG AMANTADINE HCl

Hany A. Omara[a, *], Alaa S. Amin[b] and Sayed A. Shama[b]

[a] Chemistry Department, Faculty of Science, Sirt University, Sirt, Libya.

[b] Chemistry Department, Faculty of Science, Benha University, Benha, Egypt.

Article Received on
21 August 2013,

Revised on 27 Sept. 2013,
Accepted on 29 October 2013

***Correspondence for Author:**

Dr. Hany A. Omara
Chemistry Department,
Faculty of Science, Sirt
University, Sirt, Libya.
hanyomara666@yahoo.com

ABSTRACT

Two simple, rapid, accurate, and sensitive spectrophotometric methods have been developed for the determination of amantadine HCl (AMD) in pure form and in pharmaceutical formulations. The first method is based on the reaction of the drug with potassium permanganate in the presence of sodium hydroxide to produce a water-soluble bluish-green colored species measurable at 610 nm. The absorbance concentration plot is linear over the range (0.2-6.5 µg/ml). The second method is based on oxidation of the drug by potassium permanganate in acidic medium, and determination of the unreacted oxidant by measuring the decrease in absorbance using two different dyes; methylene blue (MB) and amaranth dye (AM)) at a suitable λ_{max} (662 and 520 nm), respectively. Regression analysis of Beer's law plots showed good correlation in the concentration ranges (0.1-5.0, 0.1-5.6 µg/ml), respectively. The apparent molar absorptivity, Sandell sensitivity, detection and quantitation limits were calculated for two methods. Statistical treatment of the results reflects that the proposed procedures are precise, accurate and easily applicable for the determination of amantadine HCl in pure form and in pharmaceutical preparations.

KEY WORDS: Amantadine HCl, Spectrophotometric, Redox reaction, Potassium permanganate, Pharmaceutical analysis.

INTRODUCTION

Amantadine hydrochloride, ($C_{10}H_{17}N.HCl$) an aliphatic tricyclic primary amine (pK_a=10.1), is excreted predominantly unchanged into the urine and undergoes limited metabolism in man [1]. Amantadine HCl chemically as 1-adamantanamine hydrochloride. The molecule consists of adamantane backbone that is substituted at one of the four methyne positions with an amino group. This compound is sold under the name "Symmetrel" for use both as an antiviral and an anti-Parkinsonian drug, against Asian influenza and eventually received approval for the treatment of influenza virus A [2] in adults, issued an alert to doctors not to prescribe amantadine any more for the season. Among some Asian countries, A/H3N2 and A/H1N1 resistance has reached 100% [3]. Amantadine hydrochloride is an antiviral agent used against infection with influenza type A virus and to ameliorate symptoms when administered during the early stages of infection as well as in the management of herpes zoster [4]. It has mild anti-Parkinsonism activity and thus it has been used in the management of Parkinsonism, mainly in the early disease stage. AMD is usually given by mouth as the hydrochloride salt [5]. The analytical methods reported for AMD include high-performance liquid chromatography [6, 7], liquid chromatography–mass spectrometry [8, 9], GC [10, 11], capillary electrophoresis [12], potentiometry [13], fluorimetry [14], resonance Raman spectroscopy [15], NIR-spectroscopy [16]. Due to the absence of chromophores and/or auxochromes in the amantadine molecule, it shows no distinct absorption in the UV region above 200 nm. Therefore direct spectrophotometry is not useful for its determination. Few spectrophotometric methods [17-22] have been reported for its determination. These methods were sophisticated to perform and/or time consuming.

Spectrophotometry is considered as the most convenient analytical technique in pharmaceutical analysis because of its inherent simplicity and availability in most quality control laboratories [23, 24]. However, AMD does not possess any chromophore in its molecule, which is the essential requirement for the direct or indirect spectrophotometric analysis.

The oxidation reaction between $KMnO_4$ and AMD has not been investigated yet. Therefore, the present study was devoted to explore $KMnO_4$ as an oxidizing reagent in the development of two (direct and indirect) selective and sensitive spectrophotometric methods for the determination of AMD in capsules and plasma. The present work describes two spectrophotometric methods which are superior to the reported ones, for rapidity, reproducibility, time consuming and high sensitivity. The proposed methods which used are well known for their high absorptivity and they will have been utilized for estimation of

oxidant (potassium permanganate) in alkaline or in acidic medium. Where modern and expensive apparatus such as GLC, HPLC and HPTLC are not available.

EXPERIMENTAL

Apparatus

All the spectral measurement were made using Perkin Elemer λ73B Spectrophotometer, with scanning speed 400 nm/min and band width 2.0 nm, equipped with 10 mm matched quartz cells and a Metrohm (Switzerland) pH-meter were used for pH measurements. A thermostat water bath, JOUAN, J18 Bain Universal (France) was used to carry out the temperature studies.

Material and reagents

All chemicals used were of analytical grade and all solutions were freshly prepared in doubly distilled water. Pure amantadine HCl bulk powder was obtained from Egyptian Organization for Control and Pharmaceutical Research-Egypt. Amantadine HCl working solution was prepared by dissolving 0.01 g of pure AMD in 50 ml of bidistilled water and complete to 100 ml with bidistilled water to obtain the working standard solution of 100 µg/ml, store the prepared solution at room temperature.

Aqueous solutions of (1.0×10^{-4} M) MB (Merck) and (1.0×10^{-3} M) AM (Merck) were prepared by dissolving in an appropriate weight in 100 ml bidistilled water.

A stock (5.0×10^{-4} M) solution of $KMnO_4$ (Aldrich), was freshly prepared by dissolving an accurate weight in bidistilled water, and standardized [25].

A solution of 0.2 M H_2SO_4, was prepared by adding exact volume from stock (98%) concentrated acid to bidistilled water in 500 ml measuring flask, and standardized as recorded [26].

Solutions of 0.05 M carbonate free NaOH, in 500 ml measuring flask, and standardized as recorded [27].

General procedure

The method depends on oxidation of amantadine HCl by addition of 0.01-0.65 ml AMD (100 µg/ml) to 1.0 ml of 5.0×10^{-4} M $KMnO_4$ containing 2.0 ml of 0.05 M NaOH (for first method) was heated in a thermostat water bath at 45±1 ^{o}C for 2.0 min), to produce a water-soluble bluish-green colored species which measurable at λ_{max} 610 nm, against $KMnO_4$ similarly prepared as a blank. For the second method, 0.5 and 0.6 ml of 0.2 M H_2SO_4 was

added on using MB and AM, respectively. The solution was heated in a water bath at 45±1 ^{o}C for 2.0 min, the mixture of acidic solution was cooled and 0.5 ml (1.0 x 10^{-4} M) of MB and 0.4 ml (1.0 x 10^{-3} M) of AM, was added, the volume was completed to 10 ml with bidistilled water. The decrease in color intensity of dyes was measured spectrophotometrically against a blank solution containing the same constituent except drug treated similarly, at their corresponding λ_{max} 662 and 520 nm, respectively. The concentration range was determined in each case by plotting the concentration of AMD against absorbance at the corresponding maximum wavelengths.

Stoichiometric relationship

The stoichiometry of the reaction between AMD and the oxidant at the selected conditions was established by the molar ratio method. In this method 1.0 mL of 5.0x10^{-4} M $KMnO_4$ is kept constant and variable concentrations (0.1-3.0 ml) of AMD (5.0x10^{-4} M) were added. The absorbance was measured at λ_{max} against blank solution prepared in the same manner. The absorbance values were then plotted against the molar ratio [D]/[O].

Procedure for capsule forms

Twenty capsules were carefully evacuated; their contents were weighed and finely powdered. An accurately weighed quantity of the capsule contents equivalent to 10 mg of AMD was transferred into a 100 ml calibrated flask, and dissolved in about 40 ml of distilled water. The contents of the flask were swirled, sonicated for 5 min, and then completed to volume with water. The contents were mixed well and filtered rejecting the first portion of the filtrate. The prepared solution was diluted quantitatively with distilled water to obtain a suitable concentration for the analysis.

Procedure for spiked plasma samples

Aliquots of 1.0 ml of plasma were spiked with different concentration levels of AMD. The spiked plasma samples were treated with 0.1 ml of 70% perchloric acid and vortexed for 1.0 min. The samples were centrifuged for 20 min at 13000 rpm. The supernatants were transferred into test tubes and neutralized with 1.0 M NaOH solution.

RESULTS AND DISCUSSION

First Method

The first method is based on the reaction of the drug with potassium permanganate in the presence of sodium hydroxide. The reaction takes place completely after 10 min at room

temperature 25±1 ^{o}C. To accelerate the full color developments, the reaction mixture was heated in a thermostat water bath at 45±1 ^{o}C for 2.0 min. The produced water-soluble bluish-green colored species was measured at λ_{max} 610 nm (Figure 1). The color remains constant for at least 48 h.

$2Mn^{+7}O_4^- + 2OH^- + 3AMD \longrightarrow 2Mn^{+6}O_4^{2-} + H_2O + O_2 + \text{Oxidation products}$

(Violet) (Bluish–green colored λ_{max} 610 nm)

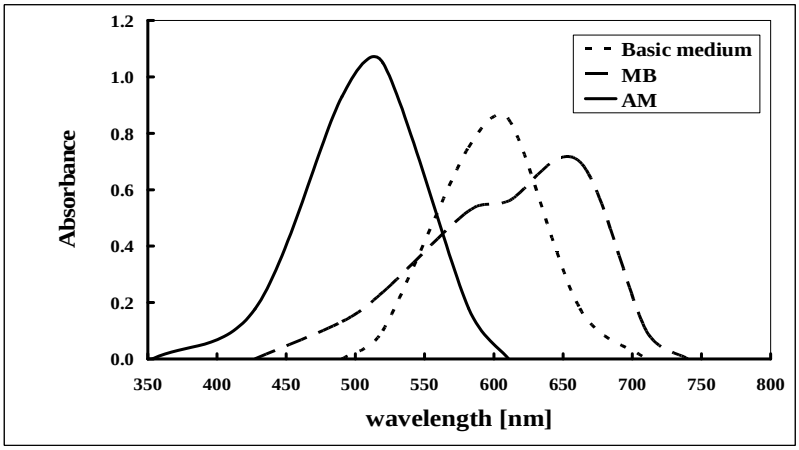

Figure 1: Absorption spectra for the reaction product of 4.0 µg/ml of AMD with 5.0 *x* 10^{-5} M $KMnO_4$, 2.0 ml of 0.05 M NaOH (for basic medium), 0.5 ml of 1.0 x 10^{-4} M (MB) and 0.4 ml of 1.0 *x* 10^{-3} M (AM), for acidic medium.

Second Method

An analytical procedure based on the specific reactivity of an amino group was investigated. The method involves two steps namely:

- Oxidation of amantadine HCl with $KMnO_4$ in acidic medium by heating in water bath 45±1 ^{o}C.
- Determination of unreacted oxidant by measuring the decrease in absorbance of dyes at a suitable λ_{max}.

$Mn^{+7}O_4^- + 8H^+ + 5e^- + 2AMD \longrightarrow Mn^{+2} + 4H_2O + \text{Oxidation products}$

(Violet) (Colorless)

$KMnO_4$ (excess) + dye $\longrightarrow$ Oxidative dye (colorless products)

The influence of each of the following variables on the reaction was tested.

Effect of permanganate concentration

The influence of potassium permanganate concentration was studied in the range from 10^{-5} - 10^{-4} M, as final concentration. The optimum results were obtained with 1.0 ml of 5.0 x 10^{-4}

M; higher concentration of $KMnO_4$ caused the color to disturbed.

Effect of medium

For first method, different concentrations of NaOH were examined. The most suitable concentration to achieve maximum yield of redox reaction was found to be 2.0 ml of 0.05 M NaOH. For the second method, different types of acid were examined (HCl, $HClO_4$, H_2SO_4, H_3PO_4, CH_3COOH and HNO_3). The most suitable acid to achieve maximum yield of redox reaction was found to be sulphoric acid. Moreover, various volumes of 0.2 M H_2SO_4 were tested and found to be 0.5 and 0.6 ml using MB and AM, respectively.

Effect of temperature and time

The reaction takes place completely after 10 min at room temperature 25±1 ^oC. The oxidation process of AMD with NaOH is catalyzed by heating in a thermostat water bath at 45±1 ^oC for 2 min, to produce a water-soluble bluish-green colored species. The oxidation process of AMD for the second method in acidic medium was catalyzed by heating in water bath of 45±1 ^oC (Figure 2). The time required to complete the reaction was 2 min. After oxidation process, the solution must be cooled at least for 1 min before addition of dye.

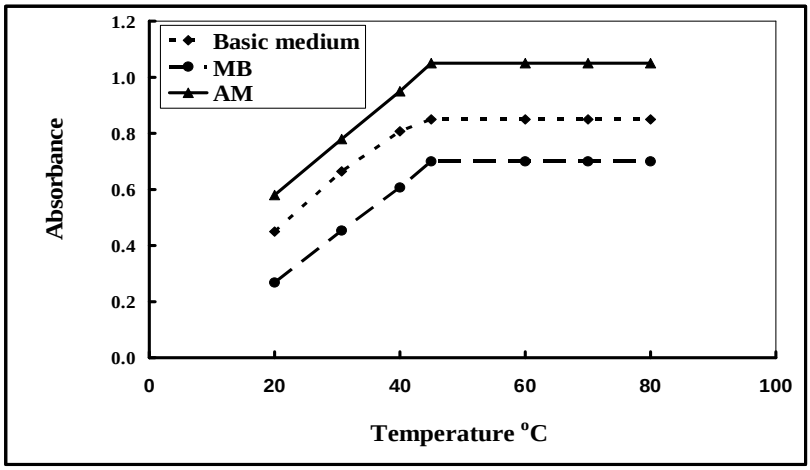

Figure 2: Effect of temperature on absorbance of 4.0 µg/ml of amantadine HCl using 5.0 x 10^{-5} M $KMnO_4$, 2.0 ml (0.05 M) of NaOH (for basic medium), 0.5 ml of 1.0 x 10^{-4} M (MB) and 0.4 ml of 1.0 x 10^{-3} M (AM), for acidic medium.

Effect of sequence of additions

The effect of sequence of additions on the oxidation process of AMD was studied by measuring the absorbance of solution prepared by different sequence of additions against a

blank solution prepared in the same manner. Experiments showed that (Oxidant-NaOH/(or)Acid-Drug) gave the best results.

Effect of dye concentration

The optimum volume of dye used for production of maximum color intensity was 0.5 ml of $1.0x10^{-4}$ M MB, or 0.4 ml of $1.0x10^{-3}$ M of AM, respectively. The effect of time after the addition of dye indicated that shaking for 1 min was sufficient to give reliable results for all dyes. The color remains constant for at least 72 h.

Stoichiometric ratio

The stoichiometry of the reaction between AMD and the oxidant at the selected conditions was established by the molar ratio method. In this method 1.0 ml of $5.0x10^{-4}$ M $KMnO_4$ is kept constant and variable concentrations (0.1-3.0 ml) of AMD ($5.0x10^{-4}$ M) were added using micropipette. The absorbance was measured at λ_{max} against blank solution prepared in the same manner. The absorbance values were then plotted against the molar ratio [D]/[O]. The stoichiometry of [D]/[O] at the selected conditions showed that the inflection of the two straight lines at 1.5 (for first method) 2.0 (for second method), respectively.

Analytical data

Beer's law limits, molar absorptivities, Sandell sensitivities, regression equations and correlation coefficients were calculated and recorded. The limits of detection (K=3) and quantitation (K=10) were established according to IUPAC definitions [28] are recorded in Table 1. In order to determine the accuracy and precision of the methods, solution containing three different concentrations of AMD were prepared and analyzed in six replicates. The analytical results obtained from this investigation were summarized in Table 2.

Interference

A systematic quantitative study was undertaken by measuring the absorbance of solutions containing 4.0 µg/ml of AMD with varying concentration of the additives and excipients such as cellulose, talc powder, lactose, calcium hydrogen phosphate, magnesium stearate, micro-crystalline cellulose and starch. Under the experimental conditions, the effect of excipients frequently found in formulations was evaluated using the proposed method; the excipients in all capsules are not interfere.

Table 1: Optical and regression characteristics of AMD for the proposed methods.

Parameters	(Method A) Basic medium	(Method B) Acidic medium	
		MB	AM
λ_{max} nm	610	662	520
Stability / h	48	72	72
Beer's law limits (µg/ml)	0.2 - 6.5	0.1 - 5.0	0.1 - 5.6
Ringbom limits (µg/ml)	0.3 - 6.1	0.2 - 4.8	0.4 - 5.1
Molar absorptivity (l mo/l. cm)	3.90×10^4	3.28×10^4	4.94×10^4
Sandell sensitivity (ng/cm)	4.81	5.71	3.80
Detection limits (µg/ml)	0.065	0.03	0.034
Quantitation limits (µg/ml)	0.196	0.098	0.105
Regression equation*: Slope (b)	0.208	0.175	0.263
Intercept (a)	0.41×10^{-3}	-2.4×10^{-3}	0.27×10^{-3}
Correlation coefficient (r)	0.9998	0.9999	0.9997
RSD** %	0.59	0.80	0.45

* A = a + bC where C is concentration of drug in µg/ml and A is absorbance.

** Relative standard deviation for six determinations.

Table 2: Evaluation of the accuracy and precision of the proposed methods for AMD.

Reagents	Taken µg/ml	Recovery %	RSD [a] %	RE [b] %	Confidence limits [c]
Basic medium (NaOH)	2.0	100.50	094	0.99	2.01 ± 0.0199
	4.0	100.25	0.57	0.60	4.01 ± 0.0241
	6.0	99.83	0.58	0.61	5.99 ± 0.0367
MB (Basic blue 9)	3.0	99.33	0.97	1.02	2.98 ± 0.0304
	4.0	100.50	0.89	0.94	4.02 ± 0.0378
	5.0	99.20	0.42	0.44	4.96 ± 0.0220
AM (Acid red 27)	3.0	100.67	0.82	0.87	3.02 ± 0.0262
	4.0	99.00	0.45	0.47	3.96 ± 0.0189
	5.0	99.60	0.64	0.67	4.98 ± 0.0336

[a] Relative standard deviation for six determinations.
[b] Relative error.
[c] 95 % confidence limits and five degrees of freedom.

Method Validation

The proposed method was successfully applied to determine AMD in its dosage forms and in spiked serum plasma. The accuracy of the proposed methods is evaluated by applying standard addition technique, in which variable amounts of the drug were added to the previously analyzed portion of pharmaceutical preparations and in spiked serum plasma. The results recorded in Table 3, were compared statistically with the official method [29] by Student's t-test (for accuracy), and variance ratio F-test (for precision) [30], at 95% confidence level as recorded in Table 4. The results showed that the t- and F- values were lower than the critical values indicating that there was no significant difference between the proposed and official methods. The proposed method was more accurate with high recoveries compared to the official method (depended on a potentiometric titration, using 0.1 M sodium hydroxide, 1.0 ml of 0.1 M sodium hydroxide is equivalent to 18.77 mg of AMD). So the proposed method can be recommended for routine analysis of AMD in pure and dosage forms in the majority of drug quality control laboratories.

Table 3: Determination of AMD in capsules using standard addition technique.

Samples	Taken µg/ml	Method A			Method B			
					(MB)		(AM)	
		Added µg/ml	Found* µg/ml	Recovery %	Found* µg/ml	Recovery %	Found* µg/ml	Recovery %
Amantine 100 mg [a]	3.0	0.0	3.02	100.66	2.99	99.66	3.01	100.33
		1.0	3.97	99.25	4.01	100.25	3.99	99.75
		2.0	4.95	99.00	5.02	100.40	4.98	99.50
		2.5	5.52	100.36			5.51	100.18
Amantadin 100 mg [b]	2.0	0.0	1.98	99.00	1.99	99.5	2.00	100.00
		1.0	2.98	99.33	3.00	100.00	2.99	99.67
		2.0	4.02	100.50	4.01	100.25	3.98	99.50
		3.0	4.96	99.20	4.98	99.60	5.02	100.40
Viraflu 100 mg [c]	2.0	0.0	2.01	100.50	2.00	100.00	1.99	99.50
		1.0	3.02	100.66	2.99	99.67	2.98	99.33
		2.0	3.94	98.50	3.99	99.75	4.02	100.50
		3.0	4.99	99.80	5.01	100.20	5.03	100.60
Spiked plasma pimples	1.0	0.0	0.99	99.00	1.01	101.00	1.00	100.00
		1.5	2.48	99.20	2.51	100.40	2.49	99.60
		3.0	4.02	100.50	3.98	99.50	3.99	99.75
		4.5	5.47	99.45			5.52	100.36

* Average of six determinations.

[a] Memphis Pharmaceutical & Chemicals Industries Company, Cairo, Egypt.

[b] Misr Pharmaceutical Industries Company, Cairo, Egypt.

[c] Sigma Pharmaceutical Industries Company, El-Monofeya, Egypt.

Table 4: Determination of amd in pharmaceutical formulations and in spiked human plasma and using the proposed and official methods.

Samples	Method A			Method B (MB)			Method B (AM)			Official method
	R* %	*t*-test	*F*-value	R* %	*t*-test	*F*-value	R* %	*t*-test	*F*-value	
Amantine 100 mg [a]	100.1	0.36	1.21	99.8	0.81	2.32	99.6	0.91	2.66	98.40
Amantadin 100 mg [b]	99.70	0.74	1.50	99.4	1.02	1.74	100.2	0.49	1.99	98.10
Viraflu 100 mg [c]	99.80	0.51	2.49	100.1	0.66	2.93	99.5	0.39	2.11	99.10
Spiked plasma pimples	100.25	1.04	2.99	99.33	0.75	1.57	100.67	0.65	2.43	98.50

* Recovery % ,

Theoretical value for t- and F- values for five degrees of freedom and 95 % confidence limits are 2.57 and 5.05, respectively.

[a] Memphis Pharmaceutical & Chemicals Industries Company, Cairo, Egypt.

[b] Misr Pharmaceutical Industries Company, Cairo, Egypt.

[c] Sigma Pharmaceutical Industries Company, El-Monofeya, Egypt.

CONCLUSIONS

The proposed method was advantageous over other reported visible spectrophotometric and colorimetric methods, related to their high reproducibility, high sensitivity, less time consuming and using simple and inexpensive reagents. Moreover, these methods allowed the determination of AMD up to 0.1 µg/ml, in addition to simplicity, rapidity, precision and stability of colored species for more than 72 h. The proposed method may be applied for routine analysis and in quality control laboratories for the quantitative determination of the AMD in raw materials and in pharmaceutical formulations.

REFERENCES

1. Aoki FY and Sitar DS, Clinical pharmacokinetics of amantadine hydrochloride. Clinical Pharmacokieitics, vol. 1988; 14, PP. 35-51.
2. Hounshell DA and Smith JK, "Science and Corporate Strategy": Du Pont R & D, Cambridge University Press, 1988; 469.
3. Varough DM, Xiyan X, Bright A, Nancy J and Alexander I. Surveillance of resistance to adamantanes among influenza A(H3N2) and A(H1N1) viruses isolated worldwide. J. of Infectious Diseases. 2007; 196: 249-57.
4. Prud'homme IT, Zoueva O and Weber J, Amantadine susceptibility in influenza A virus isolates: determination methods and lack of resistance in a Canadian sample. Clin. and Diag. Viro., 1997; vol. 8, pp. 41-51.
5. Martindale, The Complete Drug Reference Pharmaceutical Press, London, 2002; UK 33rd Edn..
6. Yoshida H, Nakao R, Matsuo T, Nohta H and Yamaguchi M. 4-(6,7-Dihydro-5,8-dioxothiazol [4,5-g] phthalazin-2-yl)benzoic acid N-hydroxysuccinimide ester as a highly sensitive chemiluminescence derivatization reagent for amines in liquid chromatography. J. of Chrom. A. 2001; 907: 39-46.
7. Suckow RF, Zhang MF, Collins ED, Fischman MW and Cooper TB, Sensitive and selective liquid chromatographic assay of memantine in plasma with fluorescence detection after pre-column derivatization. J. Chrom. B:Biomed. Scie. Appl., 1999; vol. 729, pp. 217-224.
8. Wang P, Liang Y-Z, Chen B-M, Zhou N, Yi L-Z, Yu Y and Yi Z. Quantitative determination of amantadine in human plasma by liquid chromatography–mass spectrometry and the application in a bioequivalence study. J. of Pharm. and Biomed. Anal. 2007; 43: 1519-25.
9. Arndt T, Guessregen B, Hohl A and Reis A. Determination of serum amantadine by liquid chromatography-tandem mass spectrometry. Clinica Chimica Acta 2005; 359: 125-31.
10. Sotolarova M, Houska J, Necas M and Prihoda M, Reaction of chlorodithiophosphoric acid pyridiniumbetaine with adamantane derivatives containing amino group. Polyhed., 2009; vol. 28, pp. 3985-3992.
11. Leis HJ, Fauler A and Windischhofer GW, Quantitative analysis of memantine in human plasma by gas chromatography/negative ion chemical ionization/mass spectrometry. J. of Mass Spect., 2002; vol. 37, pp. 477-480.
12. Rakestraw D, Determination of amantadine in human plasma by capillary gas

chromatography using electron-capture detection following derivatization with pentafluorobenzoyl chloride. J. of Pharm. and Biomed. Anal., 1993; vol. 11, pp. 699-703.

13. Abdel-Ghani NT, Shoukry AF and Hussein SH. Flow injection potentiometric determination of amantadine HCl. J. Pharm. & Biomed. Anal. 2002; 30: 601-11.
14. Darwish IA, Khedr AS, Askal HF and Mahmoud RM. Simple fluorimetric method for determination of certain antiviral drugs via their oxidation with cerium (IV). IL Farmaco. 2005; 60:555-62.
15. Stanic OJ, Kovalc P, Chinsky L, and Kovsky P. Pre-resonance Raman and surface-enhanced Raman spectroscopy study of the complex of the antiviral and antiparkinsonian drug amantadine with histidine. Vib. Spectr. 2001; 25: 41-51.
16. Dou Y, Sun Y, Ren Y, Ju P and Ren Y. Simultaneous non-destructive determination of two components of combined paracetamol and amantadine hydrochloride in tablets and powder by NIR spectroscopy and artificial neural networks. J. of Pharm. and Biomed. Anal. 2005; 37: 543-49.
17. Omara HA and Amin AS. Spectrophotometric microdetermination of antiparkinsonian and antiviral drug amantadine HCl in pure and in dosage forms. Arab. J. Chem. 2011; 4(3): 287-92.
18. Omara HA and Amin AS. Extractive-spectrophotometric methods for determination of anti-Parkinsonian using sulphonphthalein acid dyes. J. Saudi Chem. Soci. 2012; 16: 75–81.
19. Sultan M. Spectrophotometric determination of amantadine in dosage forms. Anal. Chem. 2004; 4: 103-09.
20. Rizk MS and Sultan M. Ultraviolet Spectrophotometric Determination of Primary Amine-Containing Drugs via Their Charge-Transfer Complexes with Tetracyanoethylene. Microchimica Acta. 2003; 143: 281-85.
21. Darwish IA, Khedr AS, Askal HF and Mahmoud RM. Simple and sensitive spectrophotometric methods for determination of amantadine hydrochloride. J. of App. Spectroscopy. 2006; 73: 792-97.
22. Mahmoud AM, Khalil NY, Darwish IA and Aboul-Fadl T. Selective spectrophotometric and spectrofluorometric methods for the determination of amantadine hydrochloride in capsules and plasma via derivatization with 1,2- naphthoquinone-4-sulphonate. Internat. J. of Anal. Chem.2009; 1: 1-8.
23. Shama SA, Amin AS, Mabrouk EM and Omara HA. Utility of oxidation- reduction reaction for the spectrophotometric determination of amlodipine besylate. Arab. J. of

Chem. 2009; 2: 59-63.

24. El-Didamoony A., Ghoneim A., Amin AS, Telbany A, Spectrophotometric determination of aminoglycoside antibiotics based on their oxidation by potassium permanganate. J. Kor. Chem. Soc., 2006; vol. 50, pp. 298-306.
25. Basset J, Denney RC, Jeffery GH and Mendham J, Vogel's Text Book of Quantitative Inorganic Analysis, 4th Edn., 1986; pp. 350.
26. Basset J, Jeffery GH & Mendham J, Vogel's Text Book of Quantitative Inorganic Analysis, 1978; pp. 308.
27. Vogel's A. I., Quantitative Inorganic Analysis 4th Edn., London, 1970.
28. IUPAC. Nomenclature, symbols, units and their usage in spectrochemical analysis II data interpretation analytical chemistry division, Spectrochim. Acta, B., 1978; 33, 241-45.
29. The British Pharmacopoeia (BP), Her Majesty's Stationary Office, London, I, 2007.
30. Miller JC and Miller JN, Statistics in Analytical Chemical 3rd Ed. Ellis Horwood Chichester, 1993.