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SPECTROPHOTOMETRIC DETERMINATION OF ANTI-PARKINSONIAN DRUG IN CAPSULES AND SPIKED PLASMA USING IRON (III) AND POTASSIUM FERRICYANIDE

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ABSTRACT

A simple, accurate, sensitive and economical procedure for the estimation of amantadine HCl (AMD), in both capsules and spiked human plasma has been developed. In the presence of potassium ferricyanide, it has been demonstrated that Fe (III) is reduced to Fe (II) by the studied drug in acidic medium. In addition, soluble Prussian blue ($KFe[Fe(CN)_6]$) was produced by the reaction between the formed Fe (II) and potassium ferricyanide, and the reaction mechanism was discussed. The absorbance of soluble Prussian blue is measured at the absorption maximum of 735 nm. Beer's law is obeyed in the concentration range 0.2-7.8 µg/mL. The molar absorptivity is 2.15 x 10^4 L/mol.cm. Sandell sensitivity is 8.73 ng/cm^2. The limits of detection as well as quantification are reported. Sex replicate analyses (n=6) of solutions containing three different concentrations of AMD was carried out. The percent error and the RSD values have been reported. The proposed method was applied to the determination of AMD in capsules and spiked human plasma and the results demonstrate that the method is equally accurate and precise as the official methods as found from the *t*- and *F*-values. The reliability of the method was established by recovery studies using standard-addition technique.

Key Words: Amantadine HCl, Spectrophotometry, Oxidation reaction, Iron (III) chloride, Potassium ferricyanide.

INTRODUCTION

Amantadine, ($C_{10}H_{17}N.HCl$) an aliphatic tricyclic primary amine, is excreted predominantly unchanged into the urine and undergoes limited metabolism in man. 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* (Hounshell DA & Smith JK, 1988) 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% (Varough *et al*., 2007).

Amantadine 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. 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 (Martindale, 2002).

The analytical methods reported for AMD include high-performance liquid chromatography (Yoshida H *et al*., 2001), liquid chromatography–mass spectrometry (Wang P *et al*., 2007; Arndt T *et al*., 2005), Capillary electrophoresis (Reichova N *et al*., 2002), Potentiometry (Abdel-Ghani N *et al*., 2002), fluorimetry (Darwish I *et al*., 2005), resonance Raman spectroscopy (Stanic O *et al*., 2001), NIR- spectroscopy (Dou Y *et al*., 2005). Due to the

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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. Spectrophotometric methods (Omara HA and Amin AS, 2011; 2012; 2013; Sultan M, 2004; Rizk M and Sultan M 2003; Sultan M *et al*., 2006; Mahmoud A *et al*., 2009) 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 (Shama S *et al.,* 2009; Shama S *et al*., 2006; Omara HA and Amin AS, 2013). Potassium ferricyanide–Fe (III) which is applied in the determination of some drugs (Li G *et al*., 2009; Wang S *et al.*, 2009; Hua Z *et al.*, 2009; Haiyan L *et al*., 2005). However, AMD does not possess any chromophores in its molecule, which are the essential requirements for the direct or indirect spectrophotometric analysis.

EXPERIMENTAL

Apparatus

All the spectral measurement were made using Perkin-Elemer model 601 UV-vis spectrophotometer with matched quartz cell of 1 cm optical length was used for spectrophotometric measurements in the wavelength range of 200-800 nm. Automatic Socorex Swiss pipettes (50-200 and 200-1000 µL) were used to measure the very small volumes. Glass micropipettes were used to measure the large volumes. A thermostat water bath, JOUAN, J18 Bain Universal (France) was used to carry out the temperature studies. A centrifuge Model 90-1 with speed 50000 rpm (USA) was used to carry out for the spiked plasma samples.

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 (Cairo, Egypt).

Amantadine HCl working solution was prepared by dissolving 0.01 g of pure AMD in 50 mL of bi-distilled water and complete to 100 mL with bi-distilled water to obtain the working standard solution of 100 µg/mL.

Aqueous solutions of 0.2% Fe (III) chloride was purchased from Merck (Darmstadt, Germany) was prepare immediately before use by dissolving 0.2g in an appropriate weight and completed to 100 mL bidistilled water.

Aqueous solutions of (0.2%) potassium ferricyanide [$K_3Fe(CN)_6$] was purchased from Merck (Darmstadt, Germany) was prepared immediately before use (freshly prepared) by dissolving an appropriate weight and completed to 100 mL bi-distilled water.

A solution of 10.0 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 (Basset J, 1978).

General procedure

Up to 0.78 mL of the standard or sample solution was transferred into a 10.0 mL calibrated flask. A 1.5 mL of 0.2% $FeCl_3$ and 1.0 mL of 10.0M sulphoric acid was added and warmed in a thermostat water bath at 35±1^{o}C for 1.0 min subsequent interaction of produced iron (II) with 1.5 ml of 0.2% potassium ferricyanide, to form Prussian blue (Dunbar K *et al.*, 1997). The volume was completed to 10.0 mL with bi-distilled water, which measurable at λ_{max} 735 nm, against acidic Fe (III)-ferricyanide similarly prepared as a blank.

Procedure for capsules forms

Twenty capsules were carefully evacuated; their contents were weighed and finely powdered. An accurately weighed quantity of the capsule contents equivalent to 10.0 mg of AMD was transferred into a 100 mL measuring flask, and dissolved in about 40 mL of distilled water. The contents of the flask were swirled, sonicated for 5.0 min; the contents were mixed well and filtered rejecting the first portion of the filtrate. The prepared solution was diluted with distilled water to obtain a suitable concentration for the analysis.

Procedure for spiked human 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 vortexes 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

Ferricyanide is the name for the anion $[Fe(CN)_6]^{3-}$. Its systematic name is hexacyanoferrate(III) ion. The most common salt of this anion is potassium ferricyanide, a red crystalline material that is used as an oxidant in organic chemistry. An analytical procedure based on the specific reactivity of an amino group was investigated. The method is based on the reduction of iron (III) by the studied drug (AMD) in acidic medium and subsequent interaction of iron (II) with ferricyanide to form Prussian blue. The product exhibits λ_{max} 735 nm (Figure 1). The color remains constant for at least 24 h. The method involves two steps namely.

- Oxidation of amantadine HCl with Fe (III) in acidic medium by heating in water bath 35±1^{o}C for 1.0 min.

2Fe (III) + H^+ + AMD ⟶ **2Fe (II) + Oxidation products**

(Brownish-yellow) (Pale yellowish green color)

Determination of Fe (II) by potassium ferricyanide to form deep blue color (Prussian blue) at a suitable $\lambda_{max.}$

Products Fe (II) + $K_3[Fe(CN)_6]$ ⟶ **$Fe[Fe(CN)_6]$**

Red color Prussian blue

Optimization

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

Effect of Fe (III) concentration

The influence of Fe (III) concentration was studied using different volumes of 0.2% $FeCl_3$. The optimum results were obtained with 1.5 mL of 0.2% Fe (III) chloride; higher concentration of Fe (III) not affected absorbance (Figure 2).

Effect of acid medium

Different types of acids were examined HCl, $HClO_4$, H_2SO_4, CH_3COOH and HNO_3. The most suitable acid to achieve maximum yield of redox reaction was found to be H_2SO_4. Moreover, various volumes of 10 M acid were found to be 1.0 mL is the optimum volume due to highly concordance results.

Effect of potassium ferricyanide concentration

Various amount of 0.2% ferricyanide was studied in 10 mL calibrated flask. The optimum results were obtained with 1.5 mL of 0.2% ferricyanide; higher concentration of ferricyanide has no effect on the absorbance, the color remains constant for at least 24 h.

Effect of temperature and time

The reaction takes place completely after 5 min at room temperature 25±1^oC. The oxidation process of AMD with Fe (III) is catalyzed by heating in a thermostat water bath at 35±1^oC for 1.0 min, in acid medium and subsequent interaction of produced iron (II) with ferricyanide to form deep blue color

Effect of sequence of additions

The effect of sequence of additions on the oxidation process of AMD by measuring the absorbance of solution prepared by different sequence of additions against a blank solution prepared in the same manner. Experiments showed that [(Fe (III)-Acid-Drug)-Ferricyanide] gave the best results.

Analytical data

Beer's law and Ringbom limits, molar absorptivities, Sandell sensitivities, regression equations and correlation coefficients were calculated and recorded in (Table 1). The limits of detection (K=3) and quantitation (K=10) were established according to IUPAC definitions (IUPAC, 1978) 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 (Table 2).

Interference

A systematic quantitative study was undertaken by measuring the absorbance of solutions containing 5.0 µg/ml of AMD with varying concentration of the additives (inactive ingredients) and excipients such as, 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.

Validation method

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 (British Pharmacopoeia, 2007) by Student's t-test (for accuracy), and variance ratio F-test (for precision) (Miller JC and Miller JN, 1993), 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 0.1M sodium hydroxide, each mL of 0.1M 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 1. **Optical and regression characteristics of AMD for the proposed method**

Parameters	Proposed method
λ_{max} (nm)	735
Stability / h	24
Beer's law limits (µg/mL)	0.2 – 7.8
Ringbom limits (µg/mL)	0.4 – 7.1
Molar absorptivity (L/ mol.cm)	2.15×10^4
Sandell sensitivity (ng/cm^2)	8.73
Detection limits (µg/mL)	0.059
Quantitation limits (µg/mL)	0.189
Regression equation [a]: Slope (b)	0.1145
Intercept (a)	-0.00357
Correlation coefficient (r)	0.9999
RSD [b] %	0.40

[a] A = a + bC where C is concentration of drug in µg/ml and A is absorbance. [b] Relative standard deviation for six determinations.

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

Reagent	Taken µg/mL	Recovery %	RSD [a] %	RE [b] %	Confidence limits [c]
Ferric – Ferricyanide	2.0	101.0	0.99	1.04	2.02 ± 0.0211
	4.5	99.56	0.65	0.69	4.48 ± 0.0307
	6.5	100.15	0.48	0.51	6.51 ± 0.0329

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

Table 3. Determination of AMD in pharmaceutical formulations using standard addition technique

Samples	Taken µg/mL	Proposed method		
		Added µg/mL	Found* µg/mL	Recovery %
Amantine 100 mg [a]	2.0	0.0	2.02	101.00
		1.5	3.49	99.71
		2.5	4.52	100.44
		3.5	5.51	100.18
PK-Pmerz Cap.100 mg [b]	3.0	0.0	2.98	99.33
		1.0	3.98	99.50
		2.0	5.01	100.20
		3.0	5.95	99.20
Viraflu 100 mg [c]	4.0	0.0	4.01	100.25
		1.0	5.02	100.40
		2.5	6.47	99.54
		3.5	7.48	99.73

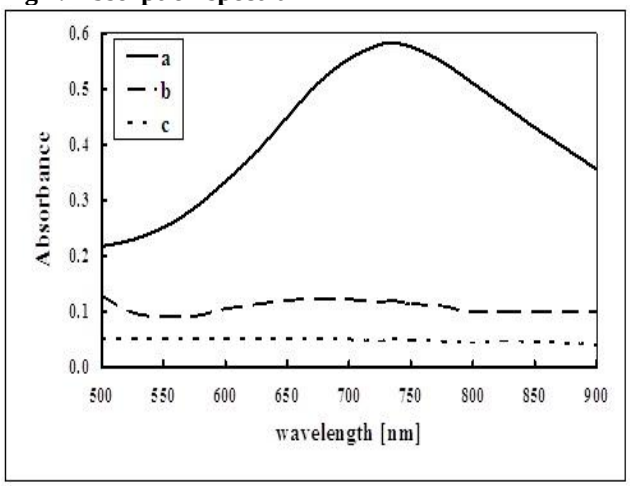
* Average of six determinations. [a] Memphis Pharmaceutical & Chemicals Industries Company, Cairo, Egypt. [b] Merz Pharma GmbH & Co. KGaA Frankfurt / Main, Germany. [c] Sigma Pharmaceutical Industries Company, El-Monofeya, Egypt.

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

Samples	Proposed method			Official method
	Recovery %	*t*-test	*F*-value	
Amantine 100 mg [a]	99.66	1.09	2.23	98.56
PK-Pmerz Cap.100 mg [b]	100.18	0.84	1.66	99.17
Viraflu 100 mg [c]	99.74	0.66	1.46	99.04
Spiked plasma pimples	100.18	0.97	2.87	98.40

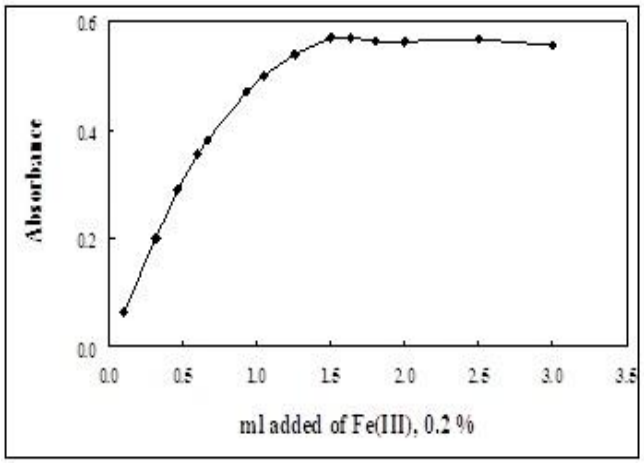
Theoretical value for t- and F- values for five degrees of freedom and 95% confidence limits are 2.57 and 5.05, respectively.
[a] Memphis Pharmaceutical & Chemicals Industries Company, Cairo, Egypt.
[b] Merz Pharma GmbH & Co. KGaA Frankfurt / Main, Germany.
[c] Sigma Pharmaceutical Industries Company, El-Monofeya, Egypt.

Fig 1. Absorption spectra



(a) $FeCl_3$–Acid–AMD–$K_3[Fe(CN)_6]$ against reagent blank
(b) $FeCl_3$–Acid–$K_3[Fe(CN)_6]$ (blank) against water (Acidic)
(c) AMD against water; AMD (5.0 µg/mL) with 1.5 mL (0.2%) iron (III), 1.0 mL (10 M) H_2SO_4, 1.5 mL (0.2%) ferricyanide.

Fig 2. Effect of 0.2% iron (III) chloride concentration; AMD (5.0 µg/mL), 1.5 mL of 0.2% ferricyanide, 1.0 mL of 10 M sulphoric acid



CONCLUSION

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 0.2 µg/mL, in addition to simplicity, rapidity, precision and stability of colored species for more than 24 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, in pharmaceutical formulations and spiked human plasma.

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