RAPID AND SIMPLE SPECTROPHOTOMETRIC METHOD FOR THE DETERMINATION OF ANTIVIRAL AND ANTI-PARKINSONISM DRUGS

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A rapid and sensitive spectrophotometric method has been developed for the quantitative analysis of three antiviral, anti-parkinsonism drugs, namely amantadine (AMA), memantine (MET), and rimantadine (RIM). The method is based on the usage of 1,3-indandione (IDO) as a chromogenic reagent to form charge transfer complexes with the studied drugs and produce colored reaction products with an absorbance maximum at 522 nm, allowing quantitative analysis of these drugs. In addition, the study was validated according to the official guidelines that permits usage in quality control laboratories. Many factors (reagent volume, diluting solvent, temperature, reaction and stability time) influencing the reactions were studied and optimized. The results showed that this method is able to detect AMA, MET, or RIM over a linear range between 10–140 μg/mL with high selectivity and robustness. Furthermore, the study results were applied to analyze the drugs in their pharmaceutical preparations with acceptable accuracy and precision.

Keywords: *spectrophotometric analysis, amantadine, memantine, rimantadine, 1,3-indandione, charge transfer complex.*

СПЕКТРОФОТОМЕТРИЧЕСКИЙ МЕТОД КОЛИЧЕСТВЕННОГО АНАЛИЗА ПРОТИВОВИРУСНЫХ И АНТИПАРКИНСОНОВЫХ ПРЕПАРАТОВ

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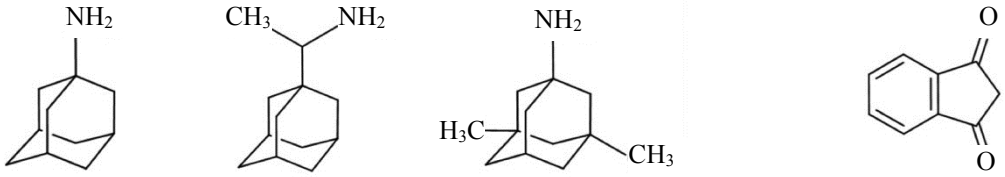
Разработан быстрый и чувствительный спектрофотометрический метод для количественного анализа трех противовирусных и антипаркинсоновых препаратов — амантадина (AMA), мемантина (MET) и ремантадина (RIM). Метод основан на использовании 1,3-индандиона (IDO) в качестве хромогенного реагента для образования комплексов с переносом заряда с исследуемыми препаратами и получения цветных продуктов реакции с максимумом поглощения на λ = 522 нм, что позволяет проводить количественный анализ этих препаратов. Подтверждено соответствие метода официальным рекомендациям, что дает возможность его использования в лабораториях контроля качества. Изучен и оптимизирован ряд факторов (объем реагента, разбавляющий растворитель, температура, время реакции и стабильность), влияющих на протекание реакций. Метод дает возможность обнаруживать AMA, MET или RIM в линейном диапазоне концентраций 10—140 мкг/мл с высокой

селективностью и надежностью. Результаты применены для анализа этих лекарственных средств в фармацевтическом составе с приемлемой точностью и воспроизводимостью.

Ключевые слова: *спектрофотометрия, амантадин, мемантин, ремантадин, 1,3-индандион, комплекс с переносом заряда.*

Introduction. Amantadine (AMA) was approved by the Food and Drug Administration (FDA) in 1966 for the treatment of viral infections, especially human influenza [1, 2]. A couple of years later, it was accidentally discovered that this drug relieved the symptoms of a Parkinsonism patient, who was originally treated with AMA for flu [3]. This newly reported finding paved the way to extensive studies that investigated the potential use of AMA for the treatment of dyskinesias, motor fluctuations, extrapyramidal syndromes, and other symptoms associated with Parkinsonism [4–7]. In 2017, the FDA has approved the use of AMA for the treatment of symptoms associated with Parkinsonism [8, 9]. While AMA was the primary drug used for the relief of Parkinsonism, other drugs that share the same chemical nucleus of AMA were developed, including memantine (MET) and rimantadine (RIM). Both drugs, MET and RIM, exhibit an equal drug efficiency with fewer side effects compared to AMA [10, 11]. In addition, MET and RIM have demonstrated a potential use for the treatment of other neurological diseases, such as dementia and Alzheimer [12, 13].



AMA MET RIM IDO

Previously reported methods for the quantification of AMA, MET, and RIM have used chromatography [14–16], electrophoresis [17, 18], electrochemical analysis [19], and nuclear magnetic resonance spectrometry (NMR) [20, 21]. Due to the lack of a prominent chromophore or fluorophore group in the chemical structure of the investigated drugs [15], relatively few spectrophotometric or fluorometric methods have been reported [22–24]. In addition, many chemicals were required for derivatization, producing spectrophotometric or fluorometric active species, or extraction prior to the analysis [25]. This indicates the need for further research to develop a rapid and simple method for the analysis these drugs.

In this study, a rapid and relatively sensitive spectrophotometric method for the analysis of AMA, MET, and RIM has been developed and optimized. The method is based on using a chromogenic reagent 1,3-indandione (IDO), to initiate the formation of charge transfer complexes with the primary amine group of the investigated drugs. This interaction produced highly colored reaction products, which can be quantitatively detected in the visible region. Furthermore, factors affecting the reaction have been studied and optimized, while the study was analytically validated according to the official guidelines to permit the use in quality control laboratories.

Experimental. Spectrophotometric measurements were carried out using 1 cm quartz cells and a double-beam UV-VIS spectrophotometer (Jenway Instruments Ltd, London, UK), connected to a computer loaded with UVWIN® software. A Sartorius handy balance H51 (Hannover, Germany) and a dry block heater with heated lids (Hanyang Scientific Equipment Co., Korea) were used.

AMA hydrochloride, MET hydrochloride, and RIM hydrochloride were purchased from Sigma-Aldrich Chemical Co. (Missouri, USA) while IDO was obtained from S.D. Fine Chem Ltd., Mumbai, India. The pharmaceutical preparations used in the present study were PK-Merz® tablets (Merz Pharma, Egypt) labeled to contain 100 mg AMA per tablet, Ravemantine® tablets (Eva Pharma, El- Egypt) labeled to contain 10 mg MET per tablet, and Virolysis® oral solution (Unipharma, Egypt) labeled to contain 150 mg RIM per 1.0 mL. All reagents and solvents used in this study were of analytical grade and were used without any further purification.

Stock standard solutions containing 1.0 mg/mL of AMA, MET, and RIM were prepared separately in methanol. A fresh solution of IDO (2.0 mg/mL) was also prepared in methanol.

General procedure and construction of the calibration curves. Aliquots of AMA (100–1400 μL), MET (100–1300 μL), and RIM (100–1200 μL) of the stock standard solutions were separately transferred into 10-mL volumetric flasks. All volumes were adjusted to 2 mL with methanol, and 1.0 mL of IDO solution

was added to each flask. The volumes were then completed to the mark with acetone, and the reaction mixtures were heated at 45ºC for 20 min. The absorbance intensity was detected at 522 nm against the reagent blank treated similarly. The calibration curves were constructed by plotting the absorbance intensity versus the corresponding drug concentrations.

Drug assay in the dosage forms. Twenty tablets of the pharmaceutical preparations of either AMA or MET were accurately weighed, finely powdered, and mixed thoroughly. A precisely weighed quantity of the powdered tablets equivalent to 100 mg of each of AMA or MET was transferred into a volumetric flask (100-mL) containing ~50 mL methanol. The flask content was sonicated for 15 min, completed to 100 mL with the same solvent, and the solution was filtered while the first portion of the filtrate was discarded. The filtrate was then diluted with methanol to 100 mL to prepare a solution containing 500 µg/mL of each drug. For the analysis of RIM in its commercial dosage form, a volume of 4.0 mL of Virolysis® oral solution was transferred into a 100 mL volumetric flask and completed to the mark with methanol. An aliquot of this methanolic solution (10 mL) was diluted to 100 mL with methanol to prepare a final concentration of 600 µg/mL of RIM.

Results and discussion. *Potential reaction mechanism and spectrophotometric spectra.* A potential interaction mechanism between the studied drugs and IDO could be postulated to the formation of charge transfer complexes between the primary amine groups of the drugs with the α-carbon atom of the reagent, as illustrated in Fig. 1. According to the suggested mechanism, the investigated drugs behave like an electron donor, while IDO is considered as an electron acceptor. Due to the 1,3-diketones of IDO, the compound is enolized, and the α-carbon atom between these two ketones becomes more acidic that permits the reaction with weak bases, e.g., the studied drugs [26, 27].

The reaction between AMA, MET, or RIM with IDO produced a purple color with an absorbance maximum at 522 nm, as shown in Fig. 2. These colored products enabled the development of a quantitative spectrophotometric method for the detection of the studied drugs in the visible region without the need for extraction or multiple reagents.

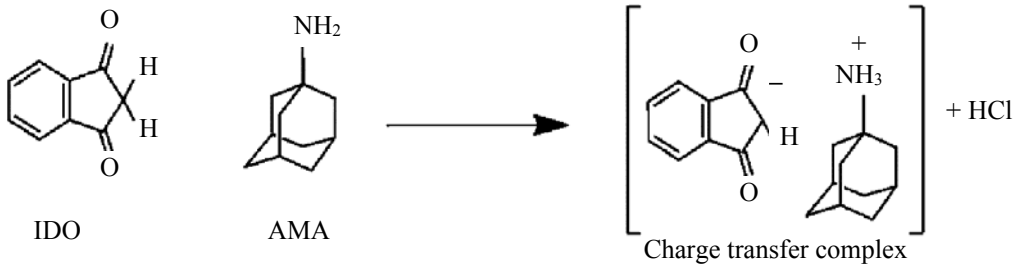


Fig. 1. The suggested reaction mechanism between AMA and IDO as an example of the formation of a charge transfer complex.

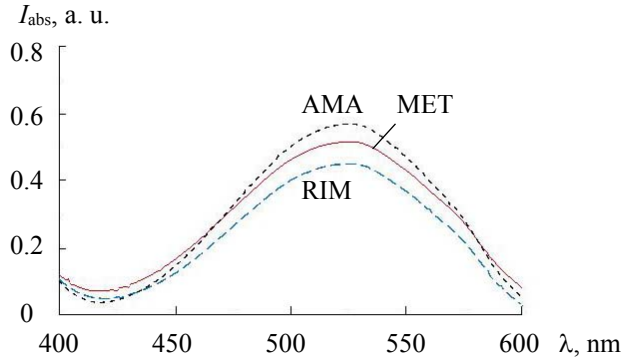


Fig. 2. The spectrophotometric spectra of the reaction products between AMA, MET, or RIM with IDO.

Variables optimization. To optimize the proposed method for quantifying AMA, MET, and RIM, many factors have been studied and optimized. These include the effect of the reagent volume, diluting solvent,

temperature, and reaction stability time. In addition, the developed method was analytically validated according to the official guidelines [28].

Effect of the reagent volume. The effect of reagent volumes on the absorption intensity of each drug-reagent product was investigated using constant amounts of AMA, MET, or RIM and different volumes of IDO (0.25–2.50 mL, 2 mg/mL). The results revealed that all reactions depended on the reagent volume, since the absorption intensity increased upon increasing the reagent volume up to 1.0 mL (Fig. 3a). A further increase in the volume above 0.75 mL was negligible. Therefore, 1.0 ± 0.1 mL of IDO (2 mg/mL) was used as the optimal reagent volume.

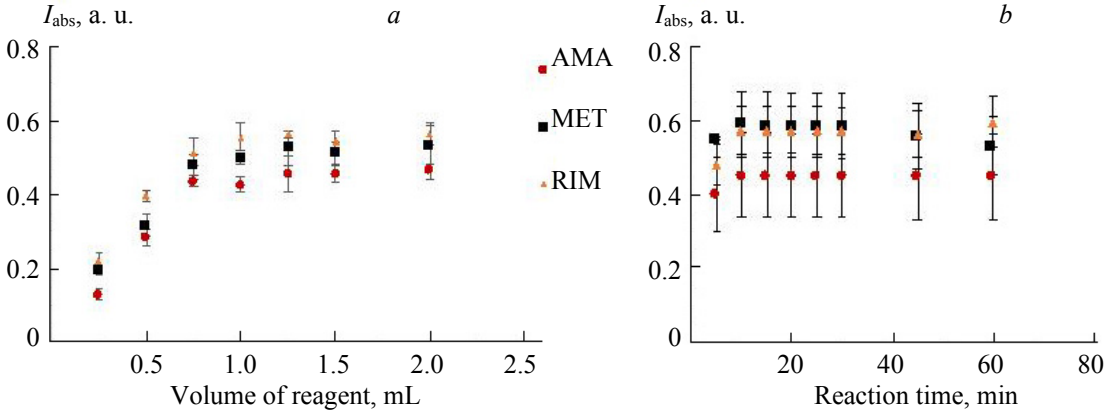


Fig. 3. The effect of different reagent volume (a) and reaction time (b) on the absorbance intensity of the reaction products of AMA, MET, or RIM with IDO, (b) heated at 45°C

Effect of diluting solvent. Different solvents, such as acetone, acetonitrile, ethanol, methanol, DMF, DMSO and chloroform, were attempted to study the effect of diluting solvent (reaction medium) on the absorbance intensity. The results showed that the max absorbance intensity for all reaction products reached a maximum when acetone was used as a medium for the reaction. Accordingly, this solvent was used as an optimized diluting solvent (medium) for all further experiments.

Effect of temperature and reaction time. The optimum temperature and reaction time for the analysis of AMA, MET, or RIM with IDO were studied at different temperatures (25–75°C) for a time period up to 1 h. The maximum absorbance intensity of the produced color was achieved after heating the reaction mixture at 45°C for 15 min, while a further increase in the heating temperature or time did not significantly influence the intensity. Therefore, heating at 45°C for 20 min was optimized for subsequent experiments. Figure 3b shows the effect of different experiment times on the interaction between each drug with IDO and heating at 45°C. In addition, the stability of the color produced was also studied, the results showed no significant decrease in the intensity of the produced color, and the reaction was almost stable for ~3 h.

Stoichiometry of the reactions. The stoichiometry of the reactions was studied using the Job's method of continuous variation using equimolar concentrations of the studied drugs and IDO [29]. The Job's plot reached its maximum value at a mole fraction of 0.5 for all reactions, which indicates that the molar reaction ratio is 1:1 for all reactions (Fig. 4).

Validation of the proposed method. The method developed for the analysis of the investigated drugs was validated using the International Conference on Harmonization (ICH) guidelines [28]. Many statistical parameters, including method linearity, range, limit of detection (LOD), limit of quantitation (LOQ), accuracy, precision, selectivity, and robustness, were detected and calculated.

Calibration curves, linearity, LOD, and LOQ. Under the optimum reaction conditions, different concentrations of AMA, MET, or RIM were analyzed using an IDO solution, and the relationship between each drug concentration and the corresponding absorbance intensity was established. The results showed a linear relationship over concentrations between 10–120 μg/mL (correlation coefficient r = 0.992), 10–130 μg/mL (r = 0.981), or 10–140 μg/mL (r = 0.993) for AMA, MET, or RIM respectively. Further, LOD and LOQ were calculated using the formulas LOD = 3σ/S and LOQ = 10σ/S, where σ is the standard deviation of the intercept and S is the slope of the calibration curve [30]. The calculated LOD and LOQ ranged from 2.40 to 3.90 μg/mL, which indicates the acceptable sensitivity of the proposed method.

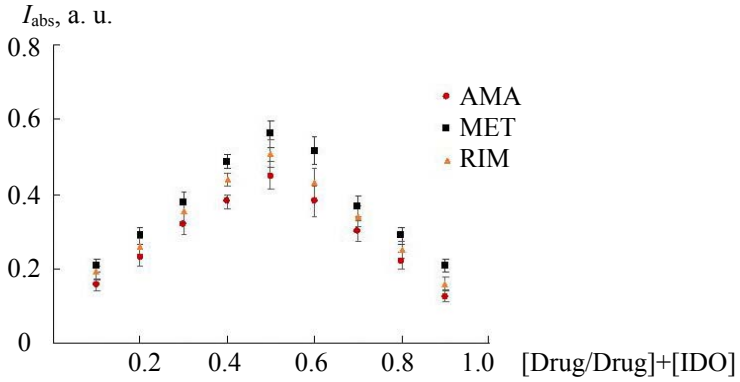


Fig. 4. Continuous variation plot for the determination of the stoichiometry of reactions.

Accuracy and precision. The accuracy and precision (inter-day and intra-day) of the proposed method were studied using a triplicate of three concentrations of AMA, MET, and RIM, within the linear range. A known amount of each drug (used amount) was used and compared with the mean for each concentration (found amount). The results are represented as the mean of the found amount ± standard deviation (RSD) as in Table 1. These results indicate the satisfactory precision and accuracy of this method to detect the investigated drugs since the value of the found amount for each drug was almost the same as the amount used. In addition, the SD values were significantly lower than the obtained mean for each concentration level of AMA, MET, or RIM, which reflects the good accuracy of the proposed method.

Selectivity and interference study. The proposed method has the advantage that all measurements can be performed in the visible region, which is outside the UV region, where pharmaceutical additives may exhibit their absorption maxima. However, a study of selectivity and interference is conducted to investigate any potential influence of common excipients that are co-formulated with AMA, MET, or RIM in pharmaceutical preparations. Additives that are commonly used to prepare tablets or oral solutions (lactose, starch, magnesium stearate, methyl paraben, sucrose, and propyl paraben) were mixed with a known amount of AMA, MET, or RIM. The results represented in Table 2 indicate that the proposed method is able to detect the studied drugs with high selectivity, since the recovered drug amount was almost the same as the amount used.

TABLE 1. The Method Accuracy and Precision Found Using Three Different Concentrations of Each Drug. The Used Amount of Each Drug is Compared to the Mean of the Amount Found after the Analysis Using the Proposed Method

Drug	Parameter	Used amount, µg/mL	Found amount ± SD, µg/mL
AMA	Inter-day	20	19.68 ± 0.21
		50	49.56 ± 0.42
		100	100.24 ± 1.36
	Intra-day	20	19.92 ± 0.22
		50	50.26 ± 0.62
		100	99.42 ± 1.22
MET	Inter- day	20	20.34 ± 0.21
		50	49.70 ± 0.30
		100	99.86 ± 1.22
	Intra-day	20	19.54 ± 0.24
		50	50.24 ± 0.46
		100	99.84 ± 1.12
RIM	Inter-day	20	19.54 ± 0.24
		50	50.24 ± 0.46
		100	99.34 ± 1.12
	Intra-day	20	19.62 ± 0.19
		60	59.42 ± 0.86
		120	119.56 ± 1.23

TABLE 2. Effect of Commonly Used Inactive Excipients on the Absorbance Intensity (% Recovery* ± SD) of the Reaction Products between the Studied Drugs and IDO

Excipient added	AMA	MET	RIM
Starch	99.24 ± 0.45	98.22 ± 0.81	98.76 ± 0.73
Sucrose	98.38 ± 0.73	99.59 ± 0.82	99.19 ± 0.65
Lactose	99.51 ± 0.93	101.55 ± 1.10	98.45 ± 0.68
Magnesium stearate	98.44 ± 1.33	98.32 ± 0.94	100.19 ± 0.55
Methyl paraben	97.85 ± 0.97	99.29 ± 0.56	98.69 ± 0.68
Lactose	99.24 ± 1.14	99.36 ± 1.46	99.08 ± 0.92
Propyl paraben	96.42 ± 1.32	98.87 ± 1.22	99.36 ± 1.16

* Average of three determinations.

Robustness. The robustness of the method was examined to evaluate the influence of small variations in the selected experimental parameters on the reaction sensitivity. A slight change in the study parameters (the reagent volume and the reaction time) did not significantly influence the absorbance intensity of the reaction products formed during the interaction of AMA, MET, or RIM with IDO (Table 3). This indicates the reliability of the proposed method for routine application in quality control laboratories.

TABLE 3. The Robustness of the Proposed Method for the Analysis of AMA, MET, or RIM

Parameter variation	AMA	MET	RIM
Reagent volume (mL)			
1.10	98.98 ± 0.71	99.07 ± 0.89	99.30 ± 0.88
9.90	98.94 ± 0.72	99.01 ± 0.62	99.20 ± 0.69
Working wavelength (nm)			
519	98.93 ± 0.81	99.10 ± 0.40	99.50 ± 0.96
525	99.01 ± 0.59	98.86 ± 0.88	99.20 ± 0.64
Reaction time (min)			
10	99.86 ± 0.86	98.30 ± 0.60	99.50 ± 0.46
20	98.40 ± 0.52	99.66 ± 0.48	99.20 ± 0.52

* Average of three determinations.

Assays in the dosage forms. The developed method was applied to detect AMA, MET, or RIM in their commercially available dosage forms. The results (percentage of recovery ± SD) were compared with those obtained by previous methods using the *F*-test and Student's *t*-test (at 95% confidence level) (Table 4). These results show that our method is able to determine the investigated drugs (AMA, MET, or RIM) in their corresponding pharmaceutical dosage forms (PK-Merz® Tablets, Ravemantine® Tablets, Virolysis® Oral solution) without any interference from the commonly added excipients. Furthermore, there is no significant difference between the results obtained in this study and those previously reported, which indicates that the developed method can be used to analyze such drugs in pharmaceutical preparations with acceptable accuracy and precision.

TABLE 4. Analysis of the Studied Drugs in their Pharmaceutical Preparations using the Proposed and Reported Methods [31, 32]

Pharmaceutical product	Ingredient (content, mg)	%Recovery* ± SD			
		Proposed method	[31,32]	*F*-value [a]	*t*-test [b]
PK-Merz® (Tablets)	AMA (100)	99.78 ± 0.81	98.20 ± 0.70	1.53	1.64
Ravemantine® (Tablets)	MET (10)	99.76 ± 0.94	99.22 ± 0.80	2.52	1.44
Virolysis® (Oral solution)	RIM (150)	98.76 ± 0.72	98.30 ± 0.97	3.20	1.06

* Average of six determinations ± SD.

[a] Relative standard deviation.

[b] Theoretical values at a confidence limit of 95%; tabulated $F = 5.05$, $t = 2.228$.

Conclusions. A simple and rapid spectrophotometric method has been developed to determine amantadine (AMA), memantine (MET), and rimantadine (RIM). The method is based on the formation of charge transfer complexes between the primary amine group of the drugs and 1,3-indandione (IDO) to form colored products that exhibit absorbance spectra at 522 nm. The method is able to detect the studied drugs over the range of 10–140 μg/mL with acceptable accuracy and precision, as well as high selectivity. Furthermore, the method has been analytically validated according to the official guidelines and extended to detect the drugs in their pharmaceutical preparations, which allows its application in quality control laboratories.

Conflict of interests. The authors confirm that there is no conflict of interest and funding organization for this research.

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