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A COMPARATIVE STUDY OF ZERO-ORDER AND DERIVATIVE SPECTROPHOTOMETRIC METHODS FOR THE DETERMINATION OF RIVASTIGMINE IN SINGLE DOSAGE FORM

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ABSTRACT

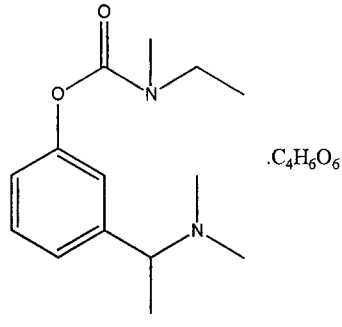
In this study zero-order , first and second derivative spectrophotometric methods were applied to the determination of Rivastigmine (RVT) in capsules. UV-spectrum, first derivative and second derivative spectrums of RVT were plotted in the spectral range of 230–280 nm. Zero-order, first derivative and second derivative absorbance values were measured at maximum wavelenghts for this three methods. Lineer calibration graphs were found in the working range of 0.2 mM – 1.0 mM (80 – 400 μg ml^{-1}). All three methods were statistically compared with each other and significant results were obtained.

1. INTRODUCTION

Alzehimer's disease (AD) is characterised by a slow decline in memory and behaviour reviewed by Cutlar and Sramek 2001 [1]. AD is a common neurogenerative disease that affects cognitive function in the elderly. Efforts to treat AD have focused on compounds that elevate cholinergic activity such as cholinesterase inhibitors and direct acting muscarinic and nicotinic agonists. AD therapies which are only FDA-confirmed slow the turnover of the neurotransmitter acetylcholine in synapse, are a group of acetylcholine esterase inhibitors. Acetylcholinesterase (AchE) enzymes degrade acetylcholine in synaps have been a great interest. Several AchE inhibitors effectively inactivate these molecules, however these inhibitors do not inhibit those neurons damaged in AD patients. Consequentely, inactivation of AchE occurs and seems likely to contribute to many adverse events like vomiting and nausea associated with these compounds. It is ideal that the inactivation of AchE would result increased Ach in the synaptic cleft and as a consequence a normal neural activity would be established in the vicinity of the damaged neurons. The drugs approved for AD treatment in U.S. , are all

acetylcholinesterase inhibitors and more recently approved one was rivastigmine[2] such drugs have effect on central cholinergic transmission [3].

Rivastigmine (ENA 713, Exelon^{R}) , (S)-N-ethyl-3-[(1-dimethyl-amino) ethyl]-N-methyl-phenyl-carbamatehydrogentartrate, is a brain selective acetylcholinesterase inhibitor of the carbamate type[4,5]. It has emprical formula of $C_{14}H_{22}N_2O_2 \cdot C_4H_6O_6$ (hydrogen tartrate salt.



Rivastigmine (RVT) displays specificity for central AchE over peripheral AchE or butyrylcholinesterase[6] is a pseudo-irreversible AchE inhibitor, has shown to delay the progression of the neuropsychological symptoms of AD[7]. It may have a benefit in treating AD. The patients showed characteristic adverse effects such as headache, nausea, vomiting and diarrhea in RVT usage during titration to the optimum dose[8,9]. It was shown that these adverse effects could be prevented by the use of antiemetics during RVT treatment [10]. It should be noted that these drugs modulate Ach levels in synapse, but don't repair damaged neurons, therefore are not effective at reversing the course of the disease.

Pharmacopoeia does not give any official determination method for RVT. Chromatographic procedures for the determination of RVT have been described, but these methods were all used for the analysis of RVT and its major metabolite in biological fluids. For example , LC-MS-MS method have been used to quantite RVT and its chief metabolite, NAP 226-90, in plasma[11]. However no analytical assays for pharmaceutical forms of this drug have been reported to date. There was no observed method for the determination of RVT bulk and RVT capsules. The aim of this work was to develop sensitive,selective and validated stability indicating method for determination of RVT in presence of dosage forms using different spectroscopic methods. For single component preparations, the simplest assay method involves the direct measurement of UV absorption at the maximum. RVT is relatively weak . UV absorbance measurements at low concentration(dissolution testing) will be unreliable. Fortunately , the derivative transformation of spectral data has been proved to be valuable procedure for the identification and quantitation of several drugs[12].

In this study three spectrophotometric method, direct absorbance measurement, first and second derivative method were subject to quantitative analysis of RVT in capsules. The assay results were statistically compared with each other [13].

2.MATERIAL AND METHODS

2.1. EXPERIMENTAL

2.1.1. Apparatus

The spectrophotometric analysis were performed on a Shimadzu UV-1601 PC double beam spectrophotometer using 1-cm quartz cells over the range 230-280 nm.

This spectrophotometer connected to a computer loaded with Shimadzu UVPC software was used for all the spectrophotometric measurements and treatment of data.

2.1.2. Materials and reagents

Rivastigmine hydrogentartrate was purchased from Novartis Ltd. Rivastigmine pure sample was used as received ; (purity 99.9 %) Exelon^{R} capsule containing (1.5mg) was obtained local drugstores.

All reagents and solvent used were of analytical grade and the solutions were prepared with doubly distilled water. A stock solution (0.8 mg ml^{-1}) was prepared in methanol : H_2O (60:40) and was further diluted with the same solvent to appropriate concentration. All working solutions were prepared freshly everyday.

2.1.3. Solution Preparation

Stock solution of concentrations 0.2 mM-1.0 mM (80 – 400 μg ml^{-1}). RVT were prepared in methanol: H_2O (60:40) and stored in dark bottles at +4°C. The working solutions under spectrophotometric investigations were prepared by dilution of stock solution.

The RVT does not change its concentration with time.

2.1.4. Procedures

Exelon® is supplied as capsules containing rivastigmine tartrate, equivalent to 1.5, 3, 4.5 and 6 mg of rivastigmine base for oral adminstration. 2.4 mg of Rivastigmine hydrogen tartrate is equivalent to 1.5 mg free base (Rivastigmine). Inactive ingredients are hydroxypropyl methylcellulose, magnesium stearate,

microcrystalline cellulose and silicon dioxide. Each hard-gelatin capsule contains gelatin, titanium dioxide and red and/or yellow iron oxide[6]. Ten capsules were accurately weighed (the content of one capsule is 1.5 mg Rivastigmine) and finally powdered. The correct amount of powder was dissolved in the methanol: H_2O (60:40) and by stirring this solution for about 20 min, a stock solution of 0.8 mg ml^{-1} was prepared. All the test solutions were obtained by diluting this stock solution with the methanol: H_2O (60:40). Zero-order spectra and derivative curves were recorded.

3. RESULTS AND DISCUSSION

Figure 1 indicates the UV absorption spectra of RVT in methanol: H_2O (60:40). The standart series of RVT in the concentration 80-400 $\mu g\ ml^{-1}$(0.2 mM-1.0 mM) were prepared in the above solvent mixture. The absorption spectra of the prepared standart series solutions were plotted in the wavelenght range of 230-280 nm. The recorded absorption spectra were used for calibration graphs. In present study, the direct absorbance measurement method was developed subject drugs in samples. The application of two alternative method is the aim of testing direct absorbance measurement method. The interference of excipient in capsules was not observed according to the obtained results.

3.1. Direct absorbance measurement method

The absorbance of standart series of RVT in the above concentration range were measured the peak amplitude corresponding to the maximum wavelenght, 263,6 nm (Figure 1). The measured absorbance values were plotted versus concentration and a straight line was obtained. The calculated calibration equation $y = 541{,}5.\ x - 4{,}3.10^{-3}$ was used for the determination of RVT in bulk and capsules. The obtained results were presented in table 1and table 3.

3.2 First derivative method

In this method, the first derivative spectra were calculated with a $\Delta\lambda$ =2 nm interval from the stored zero-order absorption spectra of the prepared samples in methanol: H_2O (60:40) (Figure 2). For the determination of RVT in the bulk and capsules, the calibration graphs were used, which obtained by measuring the $dA / d\lambda$ values at 265,5 nm and at 272,8 nm. Two calibration graphs were tested for synthetic mixtures. Regression equiations,correlation coefficients and relative standard deviations in the methods were shown in table 1. This method was successfully applied to the capsules selected and the results were illustrated in table 3.

3.3. Second derivative method

The second derivative spectra were plotted with a $\Delta\lambda$ =2 nm interval from the stored zero-order absorption spectra of the prepared samples in methanol: H_2O (60:40) (Figure 3). Lineer calibration graphs were obtained by measurement of the $d^2A / d\lambda^2$ values at 263,6 nm , 267,3 nm and 270,2 nm for this drug in bulk and capsules, respectively. Three calibration graphs
were tested for synthetic mixtures. Statistical analysis were done and indicated in table 1,3.

3.4. Analysis of capsule

Three rapid, simple and very cheaps spectrophotometric methods were successfully applied for the quantitative analysis of RVT in capsules. The results of capsule analysis obtained from these three methods were summarized in table 3. For a comparison, t-test was subject to assay results. A significant difference between three methods were not observed according to the test results.

4.CONCLUSION

In conclusion, these spectrophotometric methods were proposed for simultaneous determination of RVT in bulk and capsules. The validation of results obtained in these derivative spectral methods was realized by using the zero-order spectrophotometric method.
These methods can be used in routine analysis of RVT for the bulk and for the pharmaceutical preparations containing this drug. UV spectrophotometry is an advantageous method by the elimination of possible interferences from the other materials placed in the commercial formulations. As seen in the table 3, in assay results standard deviation of the direct UV absorption method was smaller than those obtained by using derivative methods. Also, these methods very easy and due to not to any separation and extraction steps table 3. The assay results obtained using these methods for commercial preparations were also compared with UV absorption spectrophotometric method. This method is used as a reference method due to absence of an official method for this drug. Summary of the assay results for commercial preparation was shown in table 3. There was no significant difference between each two methods.

The described methods are direct methods for analysis of RVT and do not need any expensive equipment. The methods can be easily applied in routine practices made in any laboratory possesing a spectrophotometer with a derivative accessory. As it was explained in the text , direct UV spectrophotometric method

exist one maxima at 263.6 nm for RVT giving opportunity for its determination by reading absorption values at this wavelenght. The determination of RVT can simply be made by reading absorbances at 265.6 nm and 272.8 nm in the first derivative spectra and by measuring $d^2A / d\lambda^2$ values in second derivative spectra of its solution at 263.6 nm, 267.3 nm, 270.2 nm. In the methods, the mean recoveries and relative standard deviations calculated for synthetic mixtures prepared in our laboratory are illustrated in table 2. Recovery results of these methods were found satisfactory.

These three methods proved to be suitable for routine analysis for the commercial pharmaceutical preparation selected. The assay results of commercial pharmaceutical formulation of all methods proposed were in agreement with each other.

ÖZET

Bu çalışmada Rivastigmin'in (RVT) kapsüllerdeki tayininde sıfırıncı, birinci ve ikinci türev spektrofotometri metodları uygulandı. 230 - 280 nm spektral bölgede Rivastigmin'in UV spektrumu, birinci türev ve ikinci türev spektrumları alındı. Bu üç metod için sıfırıncı, birinci türev ve ikinci türev absorbans değerleri maksimum dalga boylarında ölçüldü. Lineer kalibrasyon eğrileri0.2 mM – 1.0 mM (80 – 400 μg ml^{-1}) çalışma aralığında bulundu. Her üç metod da birbiriyle istatiksel olarak karşılaştırıldı ve anlamlı sonuçlar bulundu.

REFERENCES

[1] Cutler, N.R., Sramek, J.J., Prog.Neuro-Psychopharmacol. & Biol. Psychiat. 25 (2001) 27-57.

[2] Casademont, J., Miro, O.,Rodrigez-Santiago, B.,Viedma, P.,Blesa,R., Cardellach, F., J.Nerol.Sci.206 (2003) 23-26.

[3] Weinstock, M., CNS Drugs 12 (1999) 307-323.

[4] Sweetman, S.C., Martindale; The Complete Drug Reference 33.Edition, The Pharmaceutical Press, London (2002) 1424-1425.

[5] Enz,A.,Boddeke,H.,Gray,J.,Spiegel,R.,Ann NYAcad Sci 640 (1991) 272-275.

[6] Walsh,P.M.,PDR®;Physicians DeskReference®57.Edition,NovartisPharmaceuticals(2003) 2260-2266.

[7] Birks,J.,.Evans, J.G., Lakovidou,V., Tsolaki,M., [Cochrane review]. The Cochrane Library vol. 1 Oxford: Update Software (2001).

[8] Cory-Bloom, J., Anand,R., Veach,J., Int. J. Geriatr. Psychopharmacol.1(1998) 55-65.

[9] Sramek,J.J., Anand,R., Wardle, T.S., Irwin, P., Hardman,R.D., Cutler, N.R., Life Sci. 58 (1996) 1201-1207.

[10] Cutler,N.R., Hartman, R.D., Messina,J., An R.and Sramek,J.J.,Jhee,S.S., Society of Biological Psychiatry Poster Presentation (2000).
[11] Pommier, F., Frigola, R., J. Chrom. B 784 (2003) 301-313.

[12] Ojeda, C.B., Rojas,F.S.and Pavon,J.M.C.,Talanta 42 (1995) 1195-1214.
[13] The United States Pharmacopeia, 25 th Revision, The National Formulary, 25 th Revision, United States Pharmacopeial Convention, Inc., Rockville, MD (2002) 2256-2259.

Table1: Determination of RVT by applying the proposed methods

	Zero-order UV spec.method	First-order UV spec.method		Second-order UV spec.method		
	$\lambda_{263.6nm}$	$\lambda_{265.6nm}$	$\lambda_{272.8nm}$	$\lambda_{263.6nm}$	$\lambda_{267.3nm}$	$\lambda_{270.2nm}$
Calibration range	0.2-1.0 mM	0.2-1.0 mM		0.2-1.0 mM		
Regression equation • Slope	541.5	-29	-64	-25	- 21	- 31
• SE of slope	10.1	20.7	0.57	0.82	0.57	0.57
• intercept	-6.7×10^{-3}	-4×10^{-4}	-6×10^{-4}	2×10^{-4}	4×10^{-4}	4×10^{-4}
• SE of intercept	7.0×10^{-3}	3.1×10^{-4}	3.8×10^{-4}	5.4×10^{-4}	3.8×10^{-4}	3.8×10^{-4}
• Correlation coefficient	0.999	0.998	0.999	0.998	0.998	0.999
• SE of estimation	6×10^{-3}	1.9×10^{-4}	3.6×10^{-4}	7.9×10^{-4}	3.6×10^{-4}	3.6×10^{-4}

Table 2: Recovery data obtained for standart RVT solutions by proposed methods

Zero-order UV spec.method	Added amount (mM)	Found amount (mM) $\lambda_{263.6}$	Recovery* (%) $\lambda_{263.6}$
	0.2	0.202	101.0
	0.4	0.390	98.6
	0.6	0.590	98.7
	0.8	0.803	100.4
	1.0	1.000	100.0
			X : 99.7
			RSD : 0.22 %

First-order UV spec.method	Added amount (mM)	Found amount (mM) $\lambda_{265.6nm}$	Found amount (mM) $\lambda_{272.8nm}$	Recovery* (%) $\lambda_{265.6nm}$	Recovery* (%) $\lambda_{272.8nm}$
	0.2	0.201	0.197	100.2	98.5
	0.4	0.397	0.401	99.3	100.1
	0.6	0.594	0.604	98.9	100.6
	0.8	0.794	0.807	99.3	100.8
	1.0	0.994	0.995	99.4	99.5
				X : 99.4	X: 99.9
				RSD : 0.2%	RSD:0.42%

Second-order UV spec.method	Added amount (mM)	Found amount (mM) $\lambda_{263.6}$	Found amount (mM) $\lambda_{267.3}$	Found amount (mM) $\lambda_{270.2}$	Recovery* (%) $\lambda_{263.6}$	Recovery* (%) $\lambda_{267.3}$	Recovery* (%) $\lambda_{270.2}$
	0.2	0.199	0.201	0.197	99.7	100.1	98.5
	0.4	0.392	0.401	0.401	98.1	100.1	100.1
	0.6	0.593	0.591	0.597	98.7	98.5	99.6
	0.8	0.793	0.796	0.794	99.1	99.5	99.3
	1.0	0.993	0.982	1.004	99.3	98.2	100.4
				X:98.9	X:99.3		X:99.6
				RSD:0.27%	RSD:0.4%		RSD:0.34%

Table 3: Comparative studies for RVT formulations

Analysis techniques	Direct Absorbances measurement	First derivative spectrophotometry		Second derivative spectrophotometry		
Formulation[a] (capsule)	$\lambda_{263.6nm}$	$\lambda_{265.6nm}$	$\lambda_{272.8nm}$	$\lambda_{263.6nm}$	$\lambda_{267.3nm}$	$\lambda_{270.2nm}$
Mean (mg)[b]	1.53	1.52	1.49	1.48	1.47	1.46
R.S.D.(%)	0.02	0.05	0.03	0.04	0.03	0.03
Calculated t value T,theoretical (p=0.05)		0.80[c]	2.35[c]	2.30[c]	4.08[c]	3.91[c]

[a] Capsule, 1.5 mg per capsule
[b] Each value is the mean of five experiment
[c] NS , not significant

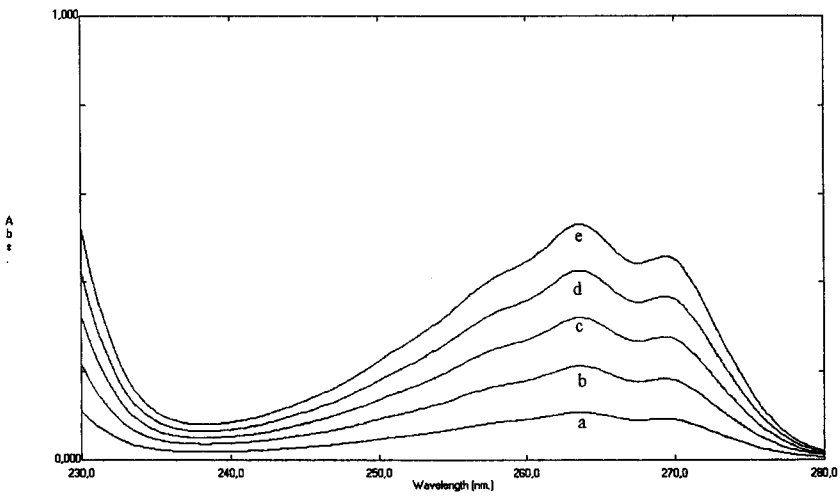


Figure 1: Zero-order absorption spectra of a) 80 $\mu g.ml^{-1}$ b) 160 $\mu g.ml^{-1}$ c) 240 $\mu g.ml^{-1}$ d) 320 $\mu g.ml^{-1}$ e) 400 $\mu g.ml^{-1}$solution of RVT in methanol:H_2O (60:40).

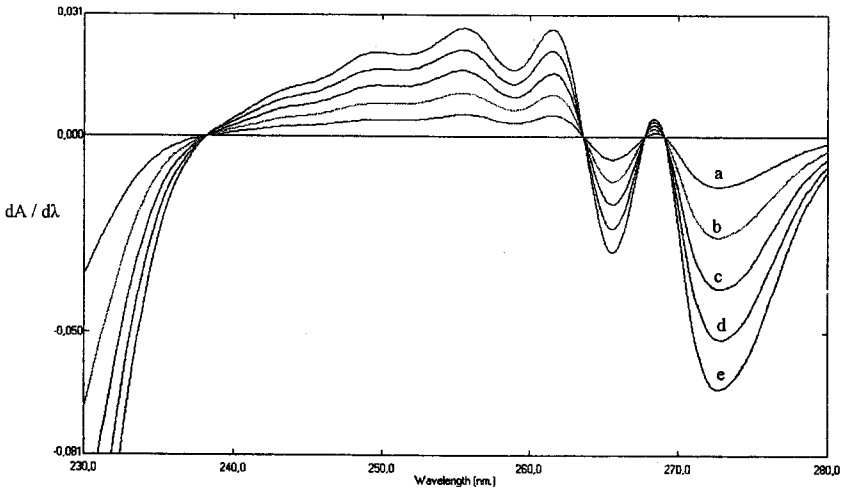


Figure 2: First derivative spectra of a) 80 μg.ml^{-1} b) 160 μg.ml^{-1} c) 240 μg.ml^{-1} d) 320 μg.ml^{-1} e) 400 μg.ml^{-1}solution of RVT in methanol:H_2O (60:40).

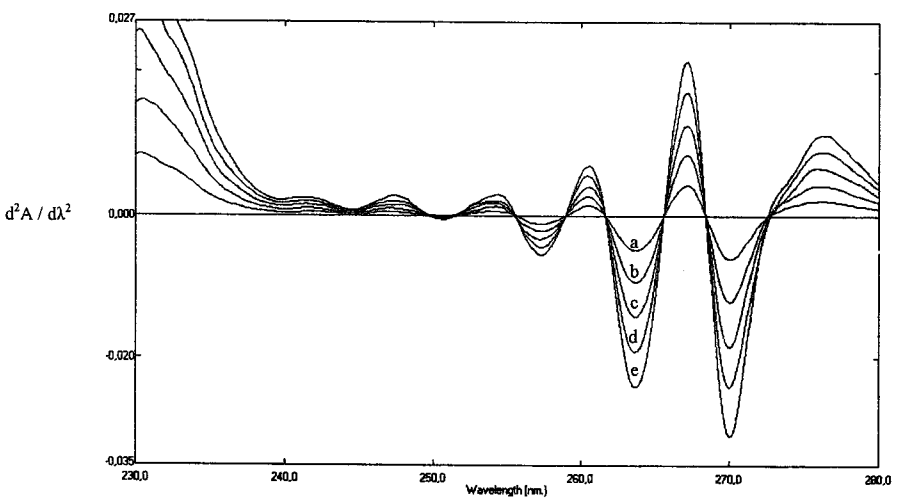


Figure 3: Second derivative spectra of a) 80 μg.ml^{-1} b) 160 μg.ml^{-1} c) 240 μg.ml^{-1} d) 320 μg.ml^{-1} e) 400 μg.ml^{-1}solution of RVT in methanol:H_2O (60:40).