Research Article

Drug Testing and Analysis

Received: 1 December 2009 Revised: 19 January 2010 Accepted: 24 January 2010 Published online in Wiley Interscience: 11 March 2010

(www.drugtestinganalysis.com) DOI 10.1002/dta.121

Spectrophotometric and spectrodensitometric methods for the determination of rivastigmine hydrogen tartrate in presence of its degradation product

Maissa Y. Salem,[a] Amira M. El-Kosasy,[b] Mohamed G. El-Bardicy[a] and Mohamed K. Abd El-Rahman[a]*

Three sensitive, selective and precise stability-indicating methods for the determination of the anti-Alzheimer's drug, rivastigmine hydrogen tartrate (RIV) in the presence of its alkaline degradation product (major metabolite, NAP 226-90) and in pharmaceutical formulation were developed and validated. The first method is a second derivative (D_2) spectrophotometric one, which allows the determination of RIV in the presence of its degradate at 262 nm (corresponding to zero crossing of the degradate) over a concentration range of 50–500 μg/ml with mean percentage recovery 100.18 ± 0.628. The second method is the first derivative of the ratio spectra (DD_1) by measuring the peak amplitude at 272 nm over the same concentration range as (D_2) spectrophotometric method, with mean percentage recovery 99.97 ± 0.641. The third method is a TLC-densitometric one, where RIV was separated from its degradate on silica gel plates using methanol : butanol : H_2O : ammonia (5 : 4 : 1 : 0.01 v : v : v) as a developing system. This method depends on the quantitative densitometric evaluation of thin layer chromatogram of RIV at 263 nm over a concentration range of 20–160 μg/spot, with mean percentage recovery 100.19 ± 1.344. The selectivity of the proposed methods was tested using laboratory-prepared mixtures. The proposed methods have been successfully applied to the analysis of RIV in pharmaceutical dosage forms without interference from other dosage form additives and the results were statistically compared with reference method.

Keywords: rivastigmine hydrogen tartrate; spectrophotometry; TLC-densitometry; stability

Introduction

Alzheimer's disease (AD) is the most common cause of dementia.[1]

Dementia is characterized by dysmnesia, intellectual deterioration changes in personality and behavioural abnormalities. It is not only a heavy burden for the patient but is also responsible for making the patient dependent on his or her family or the community. The prevalence of this disease, which mainly occurs from the sixth decade of life, increases gradually with age to reach about 30% by the end of a century of life. The neuropathology of AD is characterized by extensive neuronal cell loss, deposition of numerous senile plaques and neurofibrillary tangles in the cerebral cortex. Early neurochemical studies suggested that there is a specific loss of cholinergic neurones and/or acetyltransferase activity in AD. This led to the development of acetylcholinesterase-inhibiting drugs for treatment of AD.[1]

Rivastigmine hydrogen tartrate (Exelon), (−) *S*-*N*-ethyl-3-[(1-dimethyl-amino) ethyl]-*N*-methylphenyl-carbamate hydrogen tartrate, is a non-competitive acetylcholinesterase inhibitor of the carbamate type. Exelon is indicated for symptomatic treatment of patients with mild to moderately severe Alzheimer's dementia, as it helps to slow down the mental decline that happens in people with AD and improves their ability to cope with everyday activities.[2]

Few analytical techniques have been reported in the literature for the quantitative determination of RIV. These are GC–MS,[3,4] capillary electrophoresis,[5,6] voltammetry,[7] and potentiometry.[8] LC-MS-MS methods were also developed for simultaneous determination of RIV and its major metabolite (NAP 226-90) in biological fluids.[9–11] A stability-indicating high performance liquid chromatography (HPLC) and high performance thin layer chromatography (HPTLC) methods were also reported for the determination of RIV in bulk powder and in pharmaceutical dosage forms.[12,13]

In a modern analytical laboratory, there is always a need for significant stability-indicating methods of analysis.[14,15] An ideal stability-indicating method quantifies a drug and resolves its degradation products.[16] Reviewing the literature in hand revealed that no spectrophotometric methods concerned with the determination of RIV in the presence of its alkaline degradate, which is also a pharmacologically inactive metabolite, were reported, and no synthetic mixtures were prepared to check the specificity of other reported stability-indicating HPLC and HPTLC methods. Therefore, the aim of the present work was to develop

* *Correspondence to: Mohamed K. Abd El-Rahman, Analytical Chemistry Department, Faculty of Pharmacy, Cairo University, Kasr-El Aini Street, 11562 Cairo, Egypt. E-mail: khaled20m20@hotmail.com*

a *Analytical Chemistry Department, Faculty of Pharmacy, Cairo University, Kasr-El Aini Street, 11562 Cairo, Egypt*

b *Pharmaceutical Analytical Chemistry Department, Faculty of Pharmacy, Ain Shams University, Cairo, Egypt*

and validate stability-indicating methods for the determination of RIV in the presence of its alkaline degradate for the quality control and routine analysis of RIV in pharmaceutical preparations.

The developed methods are simple, rapid, selective, less expensive and less time-consuming compared with other published methods. These methods include second-derivative (D_2), first-derivative of the ratio spectra (DD_1) and TLC-densitometry.

Experimental

Apparatus

Spectro UV-VIS Double Beam PC, 8 scanning autocell (Culver City, California, USA LABOMED Inc., USA) serial no. Double 001137. TLC plates pre-coated with silica gel F_{254}, 0.25 mm thickness (E.Merck, Darmstadt, Germany). TLC scanner 3 densitometer model 3 S/N 130319(Camag, Muttenz, Switzerland). Camag Linomat 5 autosampler with Camag microsyringe (100 µl); (Camag, Muttenz, Switzerland). Camag TLC scanner- Model 3 S/N 130319 with winCats software (Camag, Muttenz, Switzerland). Infrared (IR) spectrophotometer – Vector 22 (Bruker Optics, Ettlingen, Germany).

Reference samples

Rivastigmine hydrogen tartrate reference standard was kindly supplied by Novartis Pharm Co. (Basle, Switzerland). Its purity was certified to be 100.13 ± 0.661.

Pharmaceutical Formulation

Exelon® capsules manufactured by Novartis Company (Basle, Switzerland). Batch no. 3003 and 4074 labeled to contain 6 mg of rivastigmine hydrogen tartrate per capsule.

Degraded samples

0.5 g of pure RIV powder was transferred to 250-ml stoppered flask, refluxed with 50 ml 0.5M NaOH with magnetic stirring for 20 min. The solution was cooled at room temperature then neutralized with 0.5 M HCl, evaporated to small volume, diluted to 10 ml with methanol; the degradation product was applied as bands on preparative TLC plates using a mixture of methanol : butanol : H_2O : ammonia (5 : 4 : 1 : 0.01 by volume) as a developing system.[8] The bands were visualized under UV light at 254 nm then scraped and the silica was suspended in the least amount of methanol, filtered and the filtrate was left to dry at room temperature (25 °C) to obtain the degradation product. The structure of the isolated degradation product was elucidated using IR spectrometry.

Materials and reagents

All chemicals used throughout this work were of analytical grade, and the solvents were of spectroscopic grade. Methanol, butanol, ammonium hydroxide 33%-Prolabo (VWR International, West Chester, PA, USA). Acetonitrile and sodium-1-heptane sulphonate; Sigma (St Louis, MO, USA). Britton-Robinson Buffer (BRB)(pH 2 – 12); prepared by mixing different volumes of 0.04 M acetic acid, 0.04 M phosphoric acid, 0.04 M boric acid and 0.2 M sodium hydroxide.

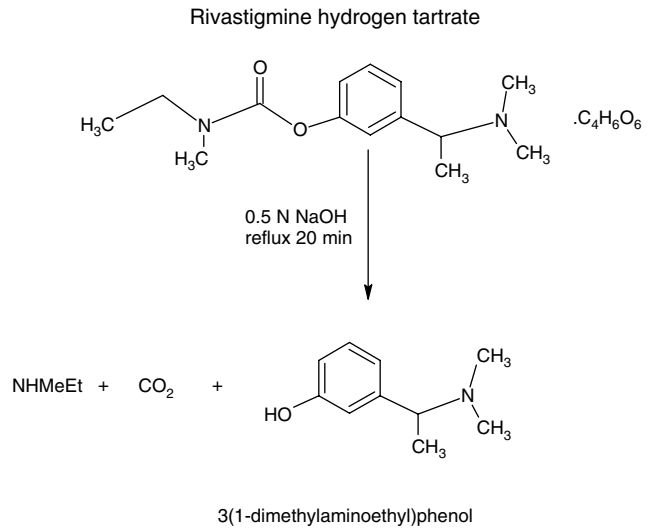

Figure 1. Alkaline degradation of RIV.[12].

Standard solutions

RIV Stock standard solution – 1 mg/ml in distilled water, for the D_2 and DD_1 methods, and 10 mg/ml in methanol for the TLC-densitometric method. Degradation product stock solution (1 mg/ml) in distilled water, for the D_2 and DD_1 methods, and 10 mg/ml in methanol, for the TLC-densitometric method.

Procedures

Construction of calibration graphs

D_2 method

Aliquots (0.5 – 5.0 ml) of RIV stock solution (1 mg/ml) were transferred into a series of 10-ml volumetric flasks, and the volume was completed with BRB (pH 7). The zero order spectra were recorded using BRB (pH 7) as a blank. The second derivative of the obtained spectra was recorded using $\Delta\lambda = 4$ nm and a scaling factor of 100. The peak amplitudes of the obtained second derivative spectra were measured at 262 nm. A calibration graph relating the peak amplitude to the corresponding concentrations of RIV was constructed, and the corresponding regression equation was computed.

DD_1 method

Aliquots (0.5 – 5.0 ml) of RIV stock solution (1 mg/ml) were transferred into a series of 10-ml volumetric flasks, and the volume was completed with BRB (pH 7). The zero order spectra of the prepared solutions were divided by the spectrum of 50 µg/ml degradation product, the first derivative of the ratio spectra (DD1) were obtained using a scaling factor of 100 and $\Delta\lambda = 4$ nm. The peak amplitudes of the first derivative of the ratio spectra were measured at 272 nm. A calibration graph relating the peak amplitudes of (1DD272) to the corresponding concentrations of RIV was constructed, and the corresponding regression equation was computed.

TLC-densitometric method

Aliquots (2 – 16 µl) from RIV stock solution were spotted (10.0 mg/ml) onto a TLC plate using Camag Linomat autosampler

with Camage micro syringe (100 µl). Spots were spaced 24.5 mm apart from each other and 15 mm from the bottom edge of the plate with a band length of 2 mm. The plates were developed in a chromatographic tanks previously saturated with the mobile phase methanol : butanol : H_2O : ammonia (5 : 4 : 1 : 0.01 by volume), by ascending chromatography. The plates were dried, spots were detected under the UV lamp (254 nm), and the plates were scanned at 263 nm. A calibration graph relating the peak to the corresponding concentration of RIV was constructed, and the regression equation was computed.

Analysis of artificial mixtures

Laboratory-prepared mixtures containing RIV and different percentages of its degradation product were prepared (Tables 2, 3) and analyzed by the proposed methods.

Application of the proposed methods to the analysis of RIV in pharmaceutical preparation

D_2 and DD_1 methods

The contents of 20 capsules were mixed and weighed. A suitable portion of powder equivalent to 100 mg RIV was transferred into a 250-ml beaker; 50 ml distilled water was added and stirred for 10 min using a magnetic stirrer then filtered into a 100-ml volumetric flask; and volume was completed with distilled water. 3 ml of the filtrate was accurately transferred to a 10-ml volumetric flask, completed to mark with BRB (pH 7). Then the procedure was completed as described with construction of calibration graphs.

TLC-densitometric method

The contents of 50 capsules were mixed and weighed. A suitable portion of powder equivalent to 250 mg RIV was transferred into

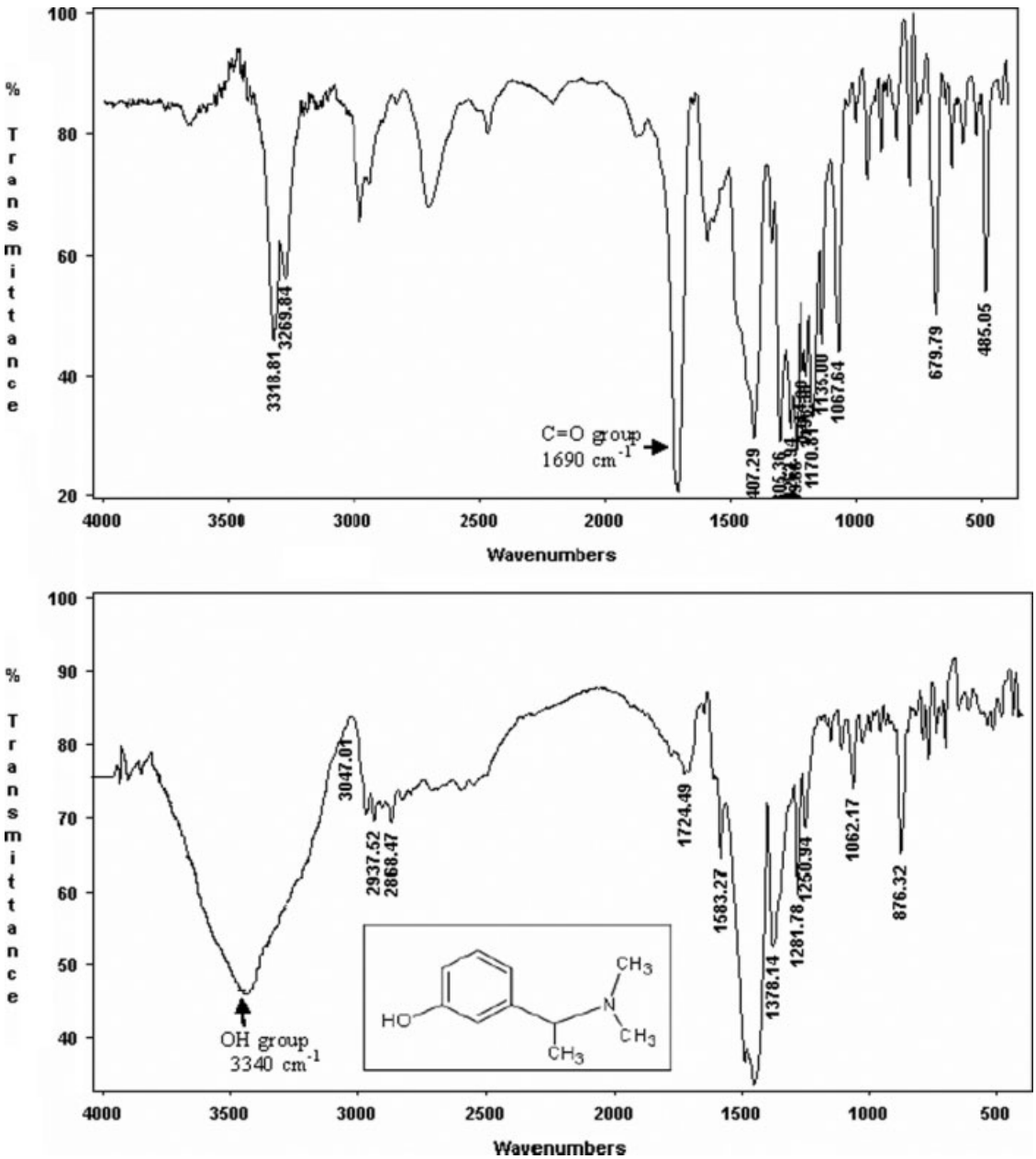

Figure 2. IR-spectra of (A) intact IV and (B) its degradation product.

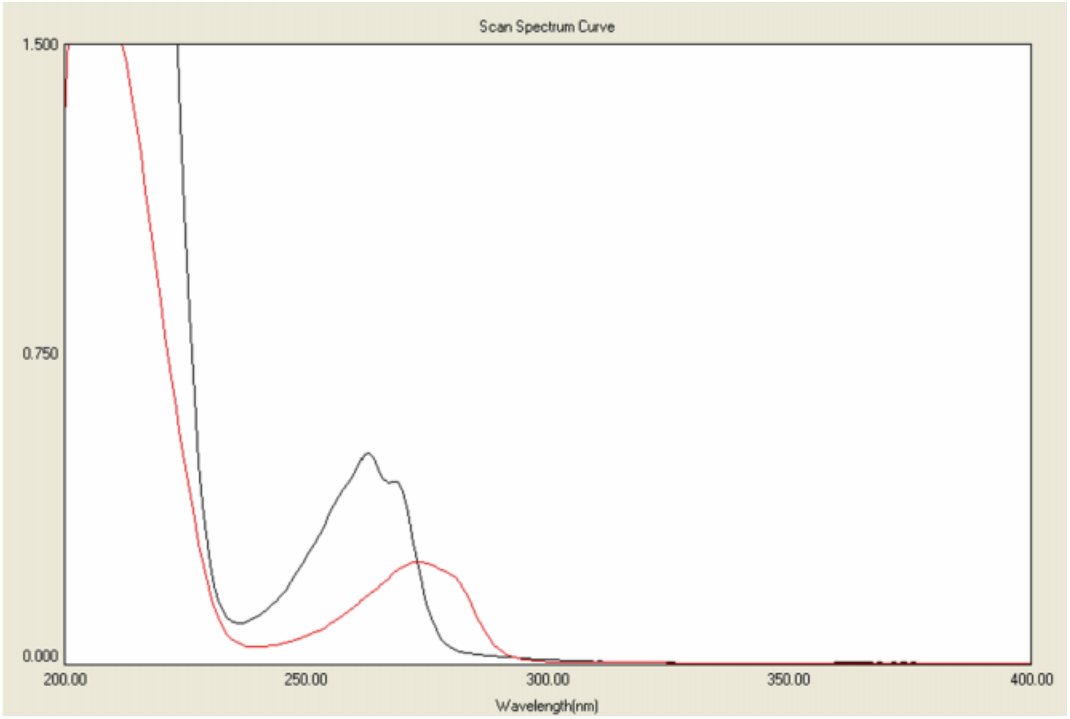

Figure 3. Absorption spectra of RIV 200 µg/ml (———) and degradation product 50 µg/ml (———) using BRB (pH 7) as a solvent.

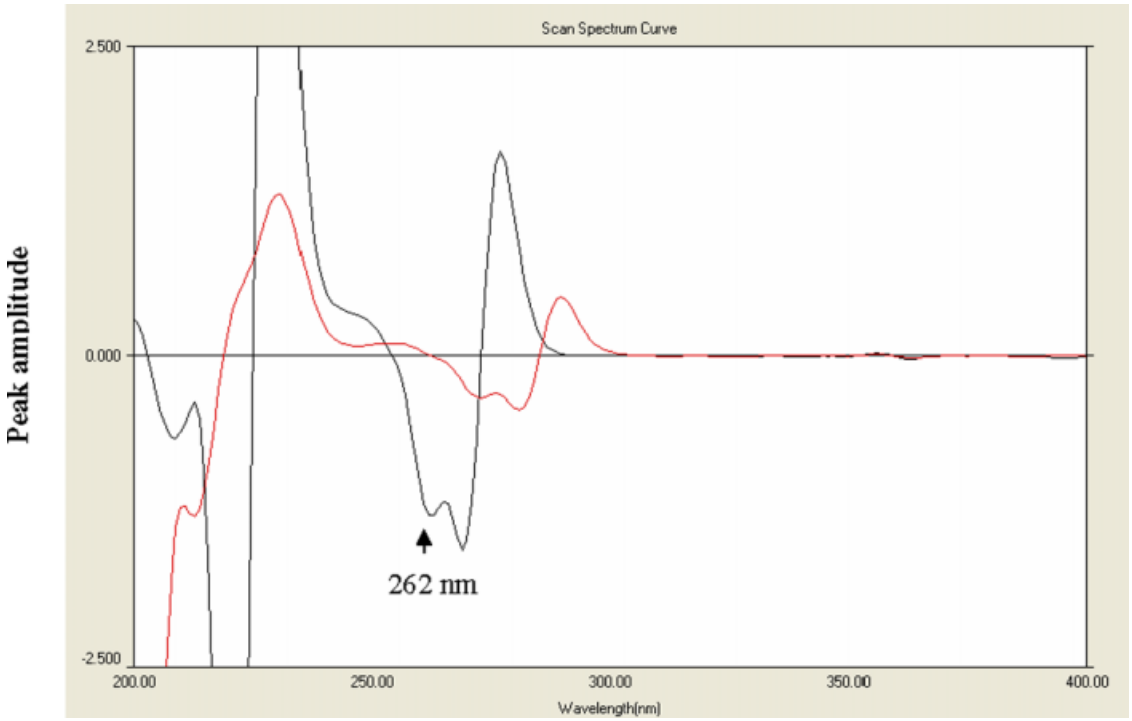

Figure 4. Second-derivative absorption spectra of RIV 200 µg/ml (———) and degradation product 50 µg/ml (———) using BRB (pH 7) as a solvent.

a 50-ml beaker; 10 ml methanol was added and stirred for 10 min using a magnetic stirrer then filtered into a 25-ml volumetric flask; the residue was washed 2 × 5 ml methanol; and then the volume was completed with the same solvent. 4.0 µl of the prepared solution was spotted in triplicate using Linomat applicator onto a TLC plate, and the procedure was completed as described with the construction of calibration graphs.

Results and Discussion

Following oral administration in humans, the plasma levels of RIV are very low due to rapid and extensive metabolism by cholinesterase-mediated hydrolysis to a weakly active decarbamylated metabolite.[17] This decarbamylated degradate was also obtained upon refluxing RIV with alkali (Figure 1); therefore the

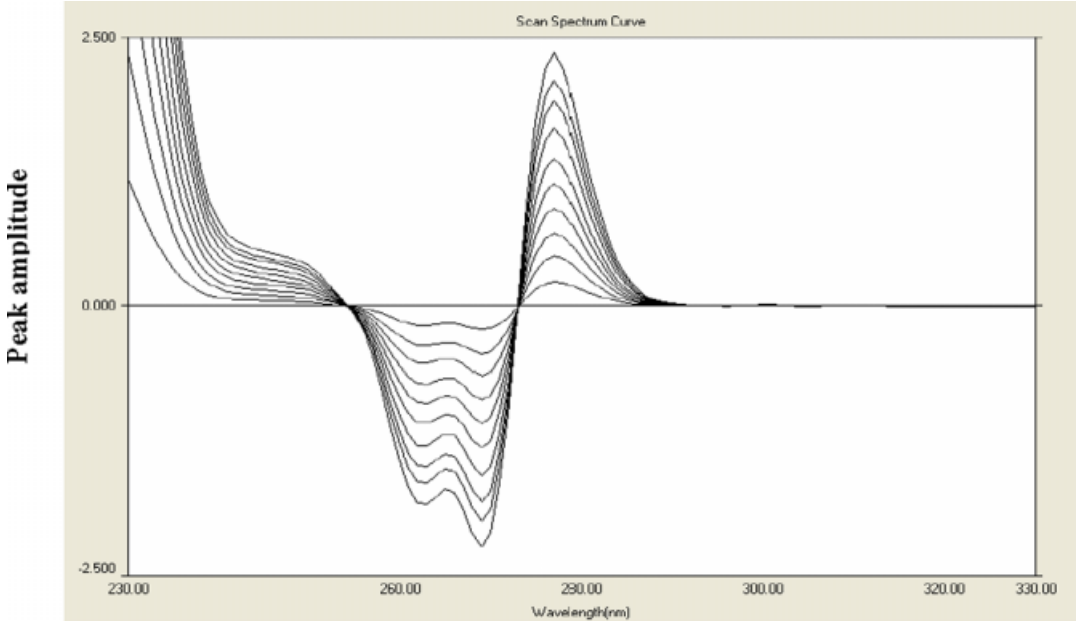

Figure 5. Second-derivative absorption spectra of 50–500 µg/ml RIV.

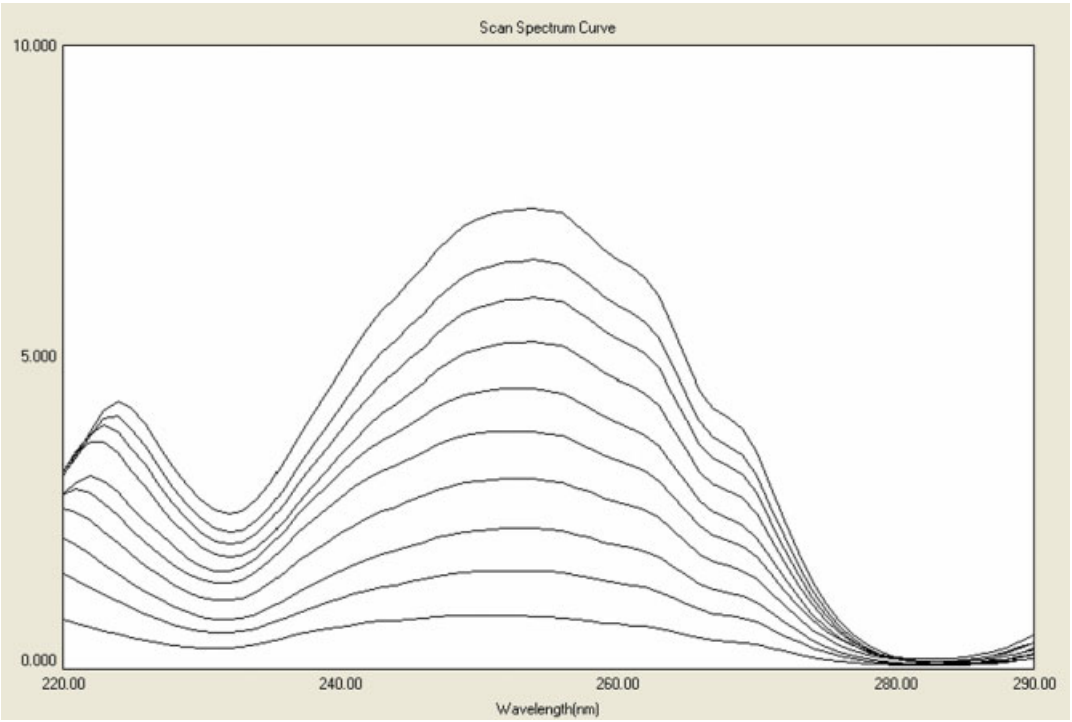

Figure 6. Ratio spectra of RIV 50–500 µg/ml using the spectrum of 50 µg/ml of degradation product as a divisor.

determination of RIV in the presence of its alkaline degradate was essential.

This was also important since the International Conference on Harmonization (ICH) guidelines entitled *Stability testing of new drugs substances and products* requires the stress testing to be carried out to elucidate the inherent stability characteristics of the active substance.[18]

The structure of the alkaline degradate was elucidated by IR spectrometry, The IR spectra of intact RIV and its alkaline degradate (Figures 2A and 2B) show that the characteristic band at 1690 cm^{-1} corresponding to C═O group in the spectrum of intact RIV (Figure 2A) disappeared in the IR spectrum of its alkaline degradate (Figure 2B), and a characteristic band at 3340 cm^{-1} corresponding to OH phenolic group appeared which confirmed the previously reported mechanism of degradation.[12]

The focus of the present work was to develop accurate, specific, reproducible and sensitive stability-indicating methods for the determination of RIV in pure form or in pharmaceutical formulations in the presence of its alkaline degradation product.

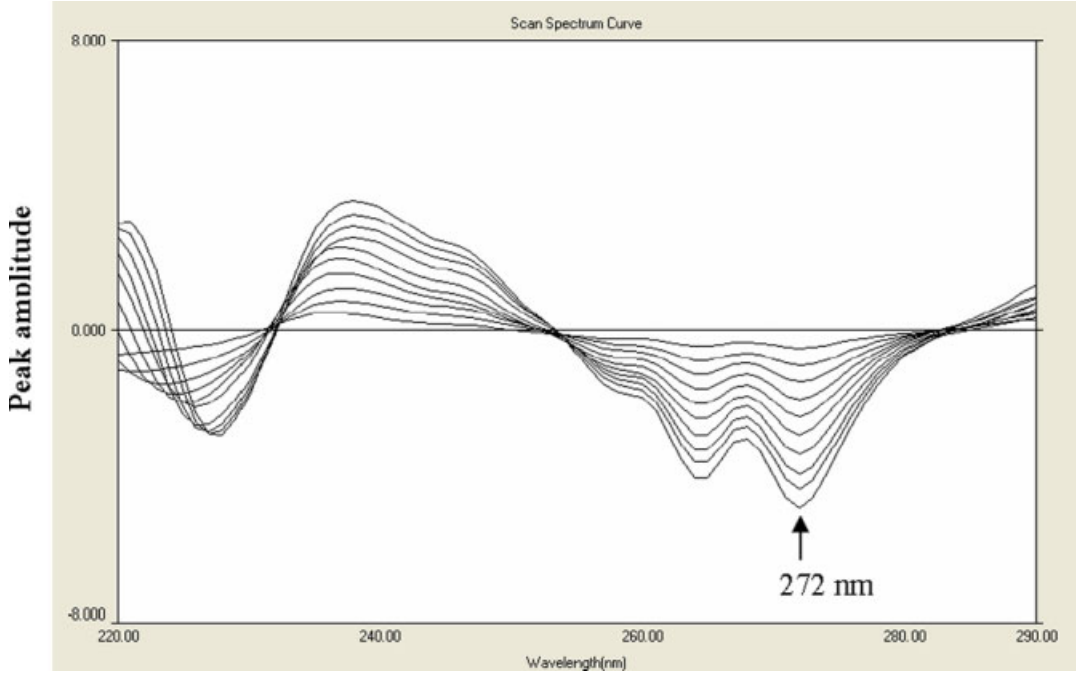

Figure 7. First derivative of ratio spectra of RIV (50–500 μg/ml) using the spectrum of 50 μg/ml of degradation product as a divisor.

Table 1. Assay validation sheet of the proposed methods for the determination of pure samples of RIV and parameters of the corresponding regression equations

	RIV		
Parameter	D_2 method	DD_1 method	TLC-densitometric method
Accuracy (mean ± SD).	100.18 ± 0.628	99.97 ± 0.641	100.19 ± 1.344
Precision repeatability[a]	100.16 ± 0.929	99.82 ± 0.939	100.69 ± 0.711
Intermediate precision[b]	99.71 ± 0.981	100.48 ± 0.781	98.87 ± 0.993
Specificity	98.45 ± 0.756	100.81 ± 1.009	99.91 ± 1.153
Linearity			
slope	−0.0052	−0.0089	0.0075
Intercept	0.0413	0.0369	0.1056
Corr. Co-eff. (r)	0.9998	0.9999	0.9997
Range	50–500 μg/ml	50–500 μg/ml	20–160 μg/spot

[a] The intraday and
[b] the inter-day mean values ± standard deviations of samples of concentration of 100, 150, 200 μg/ml of RIV.

D_2 and DD_1 methods

The zero-order absorption spectra of RIV and its alkaline degradate at neutral pH showed that RIV alkaline degradate overlaps with that of intact RIV and hinders direct spectrophotometric measurements (Figure 3). Derivative spectrophotometry is an analytical technique of great utility for extracting both qualitative and quantitative information from spectra composed of unresolved bands, and for eliminating the effect of baseline shifts and baseline tilts by using the first or higher derivatives of absorbance with respect to wavelength.[19] A rapid, simple and low-cost spectrophotometric method based on measuring the peak amplitude of D_2 spectrum of RIV at 262 nm (corresponding to zero-crossing of the degradate) was developed with good selectivity without interference of alkaline degradate as shown in Figure 4. BRB was used to adjust the pH to 7 because the alkaline degradate of RIV undergoes spectral changes due to the simple reversible ionization of the phenolic hydroxyl group at different pHs. In order to optimize the D_2 method, different smoothing and scaling factors were tested, where a smoothing factor $\Delta\lambda = 4$ and a scaling factor of 100 showed a suitable signal-to-noise ratio and the spectra showed good resolution (Figure 5).

In order to improve the selectivity of the analysis of RIV in the presence of its alkaline degradate, a DD_1 spectrophotometric method was also established. The main advantage of the method is that the whole spectrum of interfering substance is cancelled. Accordingly, the choice of the wavelength used for calibration is not critical, as in the D_2 method.

In order to optimize the DD_1 method, several divisor concentrations of 50, 100, 150 and 200 μg/ml of the degradate were tried; the best result was obtained when using 50 μg/ml of the degradate as a divisor. Different smoothing and scaling factors were tested, where a smoothing factor $\Delta\lambda = 4$ and a scaling factor = 100 were suitable to enlarge the signal of RIV to

Table 2. Determination of RIV in laboratory-prepared mixtures by the proposed D_2 and DD_1 methods

Degradation product %	Concentration (μg/ml)		D_2 method	DD_1 method
10	450.0	50.0	100.12	98.89
20	400.0	100.0	100.77	99.98
30	350.0	150.0	98.96	99.25
40	300.0	200.0	99.13	98.93
50	250.00	250.0	100.91	101.23
60	200.0	300.0	101.10	100.39
70	150.0	350.0	120.23*	99.69
80	100.0	400.0	131.62*	98.87
90	50.0	450.0	142.89*	101.17
Mean			100.17	99.82
SD			0.930	0.940
RSD%			0.928	0.942

* Rejected values.

Table 3. Determination of RIV in laboratory prepared mixtures by the proposed TLC-densitometric method

Mixture number	Concentration (μg/spot)		Recovery %
	Intact	Degradation product	
1	140.00	20.00	99.56
2	120.00	40.00	99.10
3	100.00	60.00	98.90
4	80.00	80.00	98.52
5	60.00	100.00	101.23
6	40.00	120.00	100.92
7	20.00	140.00	101.13
Mean			99.91
SD			1.153
RSD%			1.154

facilitate its measurement and to diminish error in reading the signal (Figures 6 and 7). Dividing the absorption spectra of RIV in the range of 50–500 μg/ml by the absorption spectrum of 50 μg/ml of the degradate (as a divisor), the obtained ratio spectra were differentiated with respect to wavelength. DD_1 values showed good linearity and reproducibility at 272 nm.

The linearity of the peak amplitudes of the D_2 curves at 262 nm and the peak amplitudes of the DD_1 curves at 272 nm was studied. A linear relationship was obtained in the range of 50–500 μg/ml for the drug, and the regression equations were computed (Table 1). The methods were checked by analysis of laboratory-prepared mixtures of RIV and its alkaline degradate in different ratios as presented in Table 2. RIV could be determined in the presence of up to 60% of its alkaline degradate, with mean percentage recovery of 100.18 ± 0.628 in the case of the D_2 method. While in the case of the DD_1 method RIV could be determined in the presence of up to 90% of degradate with mean percentage recovery of 99.97 ± 0.641.

TLC-densitometric method

A stability-indicating TLC-densitometric method for the determination of RIV is also described. Several trials were done to choose a developing system which can separate RIV from its degradation product including methanol : ethyl acetate : butanone (6 : 3 : 1 v : v : v) and butanol : methanol : H_2O : NH_3 (5 : 4 : 1 : 0.01 v : v : v : v). The first system was not satisfactory because it did not affect good separation of the drug and its degradation product; it separated the two spots but with R_f close to each other (R_f = 0.41, 0.35 for intact drug and degradation product, respectively). Satisfactory separation was obtained using the second

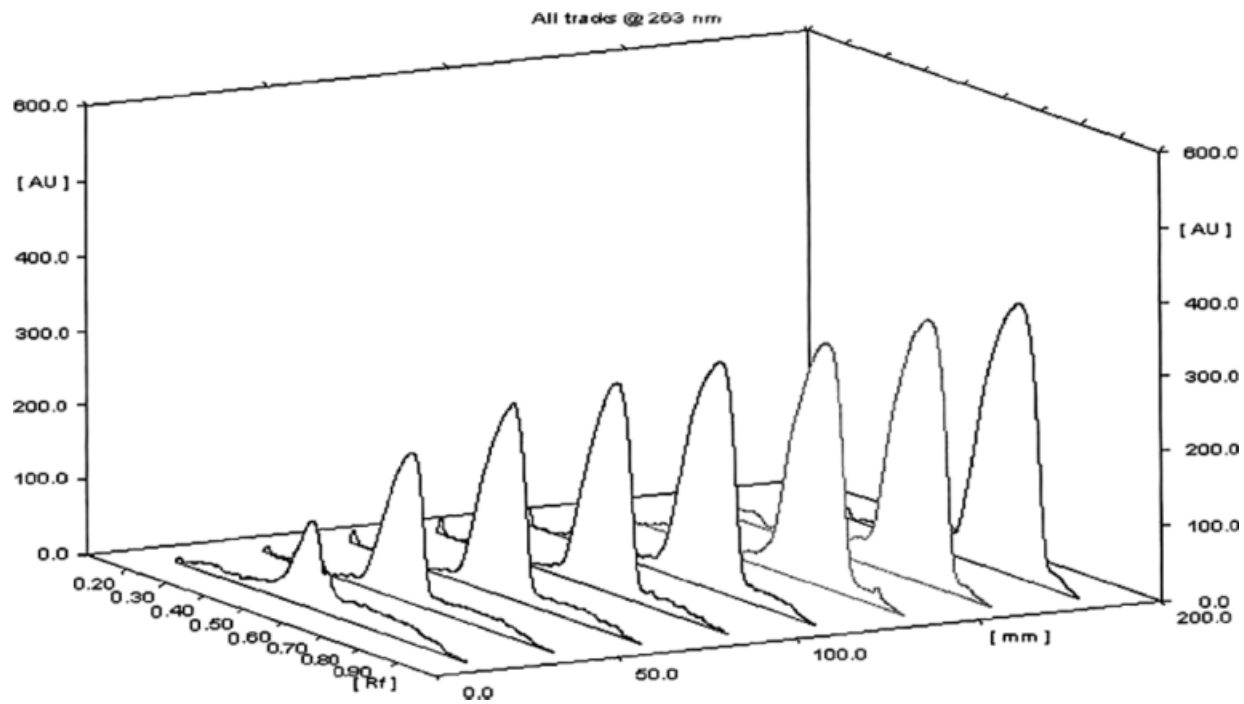

Figure 8. Scanning profile of the TLC chromatogram of RIV (20–160 μg/spot) at 263 nm.

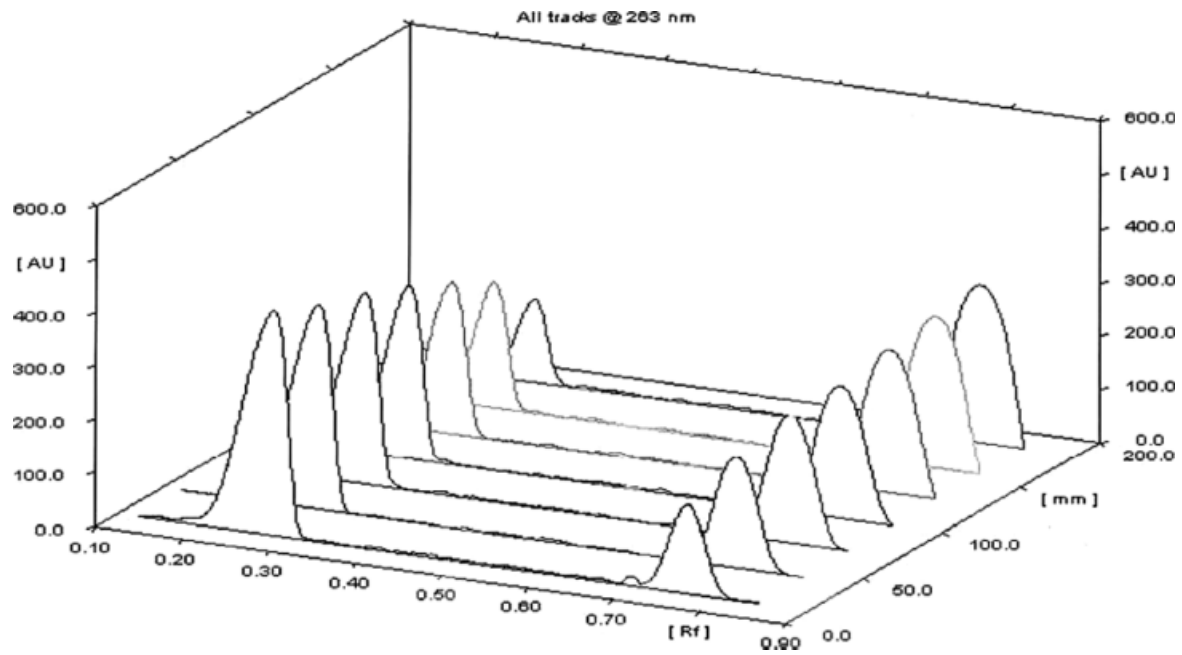

Figure 9. Thin layer chromatogram of standard RIV (20–160 µg/spot) $R_f = 0.70 \pm 0.05$ RIV and its degradation product (20–160 µg/spot) $R_f = 0.27 \pm 0.05$ using butanol : methanol : H_2O : NH_3 (5 : 4 : 1 : 0.01 by volume) as a developing system.

Table 4. Quantitative determination of RIV in Exelon® capsules by the proposed and reference methods and results of application of standard addition technique

	RIV			
	D_2 method	DD_1 method	TLC-densitometric method	Reference method[a]
Exelon® capsules	%Found ± S.D.[b]			
Batch No. 3003	99.27 ± 0.759	99.43 ± 0.656	99.71 ± 1.125	99.36 ± 0.956
Recovery of standard added %	99.66 ± 1.129	00.01 ± 0.570	100.46 ± 1.584	
Batch No. 4074	98.64 ± 0.557	99.84 ± 0.758	100.31 ± 1.003	99.71 ± 0.857
Recovery of standard added %	99.75 ± 0.793	99.51 ± 1.006	101.33 ± 0.490	

[a] HPLC method using aqueous 0.01 M sodium-1-heptane sulphonate, pH: 3.0-acetonitrile (72 : 28, v/v).
[b] Average of three determinations.

system, butanol : methanol : H_2O : NH_3 (5 : 4 : 1 : 0.01 v : v : v : v) as the mobile phase. Respective R_f values were 0.75±0.02, 0.27±0.02 for RIV and its degradate, respectively, as shown in Figure 8. This separation allows the determination of RIV at 263 nm without any interference from the degradation product as shown in Figure 9.

A linear relationship was found to exist between the integrated area under the peak of the separated spots at the selected wavelength (263 nm) and the corresponding concentration of RIV in the range of 20–160 µg/spot. The regression equation was computed (Table 1). The proposed method is valid for the determination of RIV in different laboratory-prepared mixtures in the presence of up to 87.5% of its alkaline degradate (Table 3), with mean percentage recovery of 100.19 ± 1.344.

The usefulness of the proposed methods was successfully applied for the analysis RIV in its pharmaceutical formulation and in the presence of excipients and additives was studied by assaying different batches of Exelon® capsules. The validity of the methods was assessed by applying the standard addition technique (Table 4).

Results obtained by the proposed procedures for the determination of pure samples of the drug were statistically compared to those obtained by the reference method[12] of the drug and no significant difference was observed (Table 5). Method validation was performed according to USP guidelines[20] for all the proposed methods. Table 1 shows the results of accuracy, repeatability and intermediate precision of the methods.

Conclusion

In the present work simple, sensitive, and rapid methods are described for the determination of RIV in pure form or in pharmaceutical formulations. The proposed D_2 and DD_1 spectrophotometric methods are simple, more convenient, less time-consuming and more economic stability-indicating methods compared to other published methods. The advantages of the TLC-densitometric method is that several samples can be run simultaneously using a small quantity of mobile phase unlike HPLC, thus lowering analysis time and cost per analysis and

Table 5. Statistical analysis of the results obtained by the proposed methods and the reference method[6] for the determination of RIV in pure powder form

	RIV			
Item	D_2 method	DD_1 method	TLC-densitometric method	Reference method[b]
Mean	100.18	99.97	100.19	100.25
SD	0.628	0.641	1.344	0.890
Variance	0.394	0.410	1.806	0.792
n	10	10	8	5
Student's *t*-test[b]	2.010 (2.160)	1.931 (2.160)	0.090 (2.201)	
F value[b]	0.177 (3.63)	0.703(3.63)	2.28 (6.09)	

[a] HPLC method using aqueous 0.01 M sodium-1-heptane sulphonate, pH: 3.0-acetonitrile (72 : 28, v/v).
[b] Figures between parentheses represent the corresponding tabulated values of *t* and *F* at $P = 0.05$.

providing high sensitivity and selectivity. High values of correlation coefficients and small values of intercepts validated the linearity of the calibration graphs and the obedience to Beer's Law. The RSD values, the slopes and the intercepts of the calibration graphs indicated the high reproducibility of the proposed methods. From the results obtained, we concluded that the suggested methods showed high sensitivity, accuracy, reproducibility and specificity and can be used as stability-indicating methods. Moreover, these methods are simple and inexpensive, permitting their application in quality control laboratories.

References

[1] H Braak, E Braak, in *Neurodegenerative Diseases* (Ed: D. B Calne), WB Sanders: Philadelphia, **1994**, p. 565.
[2] P Bar-on, C. B Millard, M Harel, H Dvir, A Enz, J. L Sussman, J Silman, *Biochemistry* **2002**, *41*, 3555.
[3] V. P Shah, *Pharm. Res.* **1992**, *9*, 588.
[4] Y Sha, C Deng, Z Liu, T Huang, B Yang, G Duan, *J. Chromatogr. B* **2004**, *806*, 271.
[5] A Kavalirova, M Pospisilova, R Karlicek, *Anal. Chem. Acta* **2004**, *525*, 43.
[6] Y. H Hsieh, Y. H Yang, H. H Yeh, P. C Lin, S. H Chen, *Electrophoresis* **2009**, *30*(4), 644.
[7] S Dermis, Hacettepe University, *Journal of the Faculty of Pharmacy,* **2006**, *26*(1), 1.
[8] A. M El-Kosasy, M. Y Salem, M. G El-Bardicy, M. K El-Rahman, *Chem. Pharm. Bull.* **2008**, *56*(6), 753.
[9] F Pommier, R Frigola, *J. Chromatogr. B* **2003**, *784*, 301.
[10] J Bhatt, G Subbaiah, S Kambli, B Shah, S Nigam, M Patel, A Saxena, A Baliga, H Parekh, G Yadav, *J. Chromatogr. B.* **2007:**, *852*, 115.
[11] S. V Frankfort, M Ouwehand, M. J van Maanen, H Rosing, L. R Tulner, J. H Beijnen, *Rapid Comm. Mass. Spect.* **2006**, *20*(22), 3330.
[12] B. M Rao, M. K Srinivasu, K. P Kumar, N Bhradawaj, R Ravi, P. K Mohakhud, G Om Reddy, P. R Kumar, *J. Pharm. Biomed. Anal.* **2005**, *37*, 57.
[13] A Karthik, G. S Subramanian, P Musmade, A Ranjithkumar, M Suruliverlrajan, N Udupa, *J. Planar Chromatogr. Mod. TLC* **2007**, *20*(6), 457.
[14] M. A El-Sayed, *Drug Test. Anal.* **2009**, *1*(6), 279.
[15] M. A El-Sayed, M. A Mohammad, *Drug Test. Anal.* **2009**, *1*(5), 228.
[16] A Ashour, M. A Hegazy, A. A Moustafa, K. O Kelani, L. E Abdel Fattah, *Drug Test. Anal.* **2009**, *1*(7), 327.
[17] *Clarke's Analysis of Drugs and Poisons*, 3rd edn., Pharmaceutical Press: London, **2004**.
[18] ICH, Stability testing of new drug substances and products. International Conference on Harmonization, Geneva, 27 October 1993.
[19] J. J Berzas Nevado, J Rodriguez Flores, M. J Villasenor Lierena, *Anal. Lett.* **1994**, *27*, 1009.
[20] The United States Pharmacopeia and National Formulary, *The Official Compendia of Standards, Asian Edition,* USP 30-NF 25 The United States Pharmacopeial Convention Inc.: Rockville, MD, **2007**.