ELSEVIER

Journal of Pharmaceutical and Biomedical Analysis
27 (2002) 923–932

JOURNAL OF
PHARMACEUTICAL
AND BIOMEDICAL
ANALYSIS

www.elsevier.com/locate/jpba

Spectrofluorimetric analysis of certain macrolide antibiotics in bulk and pharmaceutical formulations

Pakinaz Y. Khashaba *

Department of Pharmaceutical Analytical Chemistry, Faculty of Pharmacy, University of Assiut, Assiut, Egypt

Received 31 May 2001; received in revised form 18 July 2001; accepted 21 July 2001

Abstract

The macrolides (erythromycin, erythromycin esters, azithromycin dihydrate, clarithromycin and roxithromycin) can be analyzed by a simple spectrofluorimetric method based on the oxidation by cerium(VI) in the presence of sulphuric acid and monitoring the fluorescence of cerium(III) formed at λ_{ex} 255 nm and λ_{em} 348 nm. All variables affecting the reaction conditions as cerium(VI), sulphuric acid concentrations, heating time, temperature and dilution solvents were carefully studied. Linear calibration graphs were obtained in the range of 42.6–1200 ng ml^{-1} with a percentage relative standard deviation in the range of 0.014–0.058%. Quantitation and detection limits were calculated. The method was applied successfully for the assay of the studied drugs in pure and pharmaceutical dosage forms as tablets, capsules and suspension. Recovery experiments revealed recovery of 98.3–100.8%. The effect of potential interference due to common ingredients as glucose, sucrose, lactose, citric acid, and propylene glycol was investigated. Applying standard addition method shows a recovery of 97.7–100.9% macrolide antibiotics from their corresponding dosage forms.

Keywords: Macrolide antibiotics; Oxidation reduction; Induced fluorescence; Spectrofluorimetry; Interference study; Pharmaceutical analysis

1. Introduction

Macrolide antibiotics are produced by Streptomyces species that are used primarily against gram-positive bacteria. They are characterized by five common characteristics (a) a large lactone ring hence the name macrolide, (b) a ketone group, (c) a glycosidically linked amino sugar attached either to amino sugar or to nucleus and (d) dimethylamino moiety on the sugar residue which explains the basicity of these antibiotics (pK_a values are between 6.0 and 9.0) and affords the possibility of preparing their clinically useful salts [1]. Erythromycin (ER) has been analyzed by UV–Vis spectrophotometric methods based on reaction with an acidic dye [2,3], concentrated sulphuric acid [4], ferric ions [5], π-acceptors [6], or on the formation of blue colored complex with gentian violet at 633 nm [7] and by spectrofluorimetric methods using erythrosine B [8], or napthotriazole disulfonate [9]. Roxithromycin

* Tel.: +20-88-312-564; fax: +20-88-332-776.
E-mail address: pakinaz@yahoo.com (P.Y. Khashaba).

0731-7085/02/$ - see front matter
PII: S0731-7085(01)00609-4

(ROX) has been analyzed by spectrophotometric methods based on either ion –pair formation [10–12] or on reaction with vanillin and *p*-dimethylaminobenzaldehyde [10]. No spectrophotometric methods have been reported for the assay of azithromycin (AZ) and only one method has been described for clarithromycin (CLA) [12]. Other reported methods are mostly based on chromatographic techniques. These include TLC, paper chromatography, GC, capillary zone electrophoresis [13] and HPLC in pharmaceutical dosage forms [13–17] and in biological samples using UV detector [13,18–20], electrochemical detector [13,20–25] and fluorescence detector after precolumn derivatization [26,27]. Furthermore, many microbiological methods have been reported for their analysis [16,17,28–30], however they suffer from many disadvantages as the long incubation periods and the lack of sensitivity towards other antibiotics. Unfortunately, most official methods for the analysis of macrolides are mainly antimicrobial assays [16,17], which could be laborious, expensive and time consuming. Meanwhile some reported methods require either derivatization of the drug [26,27] or using a selective detector [14,21–26,31] which is expensive and could not be available in many laboratories.

With the exception of erythromycin, no spectrofluorimetric procedures have been described for the assay of these drugs, therefore the aim of this work was to develop a new spectrofluorimetric method for routine determination of macrolides; that is; more sensitive than already existing spectrofluorimetric assays of erythromycin [8,9], also less complex and faster than reported and official microbiological and HPLC assays [15–17]. The suggested spectrofluorimetric method depends simply on the oxidation of all the studied macrolides: erythromycin, erythromycin ester, roxithromycin, clarithromycin, and azithromycin dihydrate) with ceric ammonium sulphate (Ce(IV)) in presence of sulphuric acid and measuring the fluorescence of the resulting cerium (Ce(III)) at λ_{ex} 255 nm and λ_{em} 348 nm. Interference due to other commonly encountered excepients and additive was investigated.

2. Experimental

2.1. Apparatus

Spectrofluorimeter: SFM 23/B, Kontron, Switzerland equipped with a recorder and a 1×1 cm^2 quartz cell; was used for recording the spectra and carrying out fluorescence measurements. The calibration and linearity of the instrument were checked at frequent intervals with standard quinine sulphate (0.01 μg ml^{-1}). Wavelength calibration was performed by measuring λ excitation and λ emission of the same standard of quinine sulphate at λ_{ex} 275 nm and λ_{em} 430 nm, although no variation in the wavelength was observed. All fluorescence measurements were recorded at the lower set sensitivity.

Spectrophotometer: Uvidec-320, JASCO, Tokyo, Japan. In addition, a thermostatically controlled water-bath was used.

2.2. Materials and chemical reagents

Analytical grade chemicals were used as received. Doubly distilled water was used throughout.

Sulphuric acid: 0.25 M was prepared by mixing 13.6 ml of concentrated sulphuric acid in 1000 ml double distilled water.

Ceric ammonium sulphate (Sigma Chemie GmbH, West Germany): 0.002 and 0.003 M was prepared by dissolving 1.27 and 1.9 g in 1000 ml 0.25 M sulphuric acid, respectively. To avoid the presence of Ce(III) to some extent with Ce(IV) solutions, sodium bismuthate was added to Ce(IV) solutions to oxidize any Ce(III); if present; and excess of sodium bismuthate was eliminated by filtration [32]. The prepared solution was kept in the refrigerator and used for one week during which it is checked at frequent intervals by measuring its fluorescence intensity compared to standard solution of Ce(III), where Ce(III) was completely absent.

Roxithromycin was obtained from T3A Pharma Group (Cairo, Giza, Egypt). Erythromycin, erythromycin ethyl succinate, azithromycin dihydrate, clarithromycin, were obtained as gifts from Abbot laboratories (North

Chicago, IL). All drugs were used as received and their solutions were prepared freshly every day to be used as working standards.

2.2.1. Pharmaceutical dosage forms

Erythromycin tablets (Pyramids Medicals, by Amoun Pharmaceutical Industies Co.); and erythromycin granules (erythromycin ethyl succinate) (kahira pharm & Chem. Ind. Co. Egypt, under the licence of Abbot laboratories, North Chicago, IL): labelled to contain erythromycin stearate as 250 mg/tablet; and erythromycin as 200 mg/teaspoonful, respectively. Zithromax capsules (Pfizer, Egypt, under authority of Pfizer Inc. USA); klacid tablets (GalaxoWellcome Co., Egypt, S.A.E., El Salam city, Cairo, Egypt under licence of Abbot laboratories international); and Rulid tablets for oral suspension (Hoechst Marion Roussel, S.A.E., Egypt, under licence of Hoechst Marion Roussel, France): labelled to contain azithromycin as 250 mg/capsule; clarithromycin as 250 mg/tablet; and roxithromycin as 50 mg /tablet, respectively.

2.3. Preparation of standard solutions

An accurate weighed quantity of each of the studied macrolides was transferred into a 10-ml volumetric flask, dissolved in 5 ml absolute ethanol, sonicated for 3–5 min and then diluted to volume with the same solvent. From this solution a series of dilution was prepared quantitatively in double distilled water to obtain standard solutions having concentration ranges of: 426–4257, 477–4770, 500–12 000 and 1000–12 000 ng ml^{-1} for erythromycin, azithromycin, clarithromycin and roxithromycin, respectively.

2.4. Preparation of samples

2.4.1. Erythromycin tablets

Ten tablets were weighed and finely powdered. A weighed portion equivalent to the weight of one tablet was transferred to a 250-ml volumetric flask, sonicated for 5 min with about 100 ml absolute ethanol then the solution was completed to volume with the same solvent. The mixture was mixed well, allowed any insoluble matter to settle then filtered. A measured volume of the filtrate was diluted quantitatively with double distilled water to yield a sample solution having a concentration assumed to be 350 ng ml^{-1} of erythromycin.

2.4.2. Klacid tablets

The procedure was followed as mentioned in Section 2.4.1 to yield a sample solution having a concentration assumed to be 350 and 1000 ng ml^{-1} of erythromycin and clarithromycin, respectively.

2.4.3. Erythrocin granules for oral suspension

An accurate volume of the prepared oral suspension (200 mg 5 ml) freshly mixed and free from air bubbles was transferred to a 250-ml volumetric flask, sonicated with 100 ml absolute ethanol for 3–5 min then completed to volume with the same solvent and filtered. A measured volume of the filtrate was diluted quantitatively with double distilled water to yield a sample solution having a concentration assumed to be 350 ng ml^{-1} of erythromycin.

2.4.4. Zithromax capsules

The contents of five capsules were removed and weighed accurately. The combined contents were mixed and a portion of the powder equivalent to one capsule was transferred to a 250-ml volumetric flask. The procedure was then completed as in Section 2.4.1 to yield a sample solution having a concentration assumed to be 400 ng ml^{-1} of azithromycin.

2.4.5. Rulid tablets for oral suspension

Ten tablets were dissolved, each in one teaspoonful of distilled water, transferred quantitatively to a 500-ml volumetric flask. The combined suspension was sonicated for 3–5 min in 200 ml absolute ethanol, completed to volume with the same solvent, then filtered. A measured volume of the filtrate was diluted quantitatively with double distilled water to yield a sample solution having a concentration assumed to be 1000 ng ml^{-1} of roxithromycin.

2.5. General procedure

One milliliter of sample or standard solution was transferred by a pipette into a 10-ml calibrated flask. A volume of 1 ml of ammonium ceric sulphate (0.002 M for azithromycin dihydrate and 0.003 M for other studied macrolides) was added. The solution was mixed well and heated in a thermostatic water bath at 80 °C for 60 min for erythromycin and azithromycin dihydrate and for 30 and 45 min for roxithromycin and clarithromycin, respectively. The solution was then cooled, diluted to volume with double distilled water and measured spectrofluorimetrically at λ_{ex} 255 and λ_{em} 348 nm against a blank experiment treated similarly.

3. Results and discussion

Cerium(IV) has been used as an oxidizing agent for the determination of certain drugs as phenothiazines by monitoring the fluorescence of their sulfoxides or the Ce(III) formed. However, Ce(III) is more than four times fluorescent as the phenothiazines sulfoxides and therefore, measurement of its fluorescence can be used as a very sensitive method for the determination of these drugs [32]. In the present work macrolides were oxidized by Ce(IV) and relative fluorescence intensities of the induced Ce(III) was monitored at λ_{ex} 255 nm and λ_{em} 348 nm. To avoid interference due to the presence of Ce(III), the fluorescence intensities of Ce(IV) were measured to obtain appropriate blank correction.

3.1. Optimization of reaction conditions:

A series of experiments were conducted to establish the optimum analytical conditions for the oxidation of macrolides by Ce(IV). The parameters optimized were performed on all the studied macrolides by altering each variable in turn while keeping the others constant.

3.1.1. Effect of Ce(IV) concentration

The effect of Ce(IV) concentrations was investigated using 1 ml of different concentrations of the reagent in the range of 0.001–0.01 M employing two concentrations of the studied macrolides: 250 and 500 ng ml^{-1} for azithromycin and erythromycin; 500 and 1000 ng ml^{-1} for clarithromycin and roxithromycin. Maximum relative fluorescence intensity was obtained with a Ce(IV) concentration of 0.002 M for azithromycin and 0.003 M for other studied drugs, above which it remained constant or slightly decreased (Figs. 1

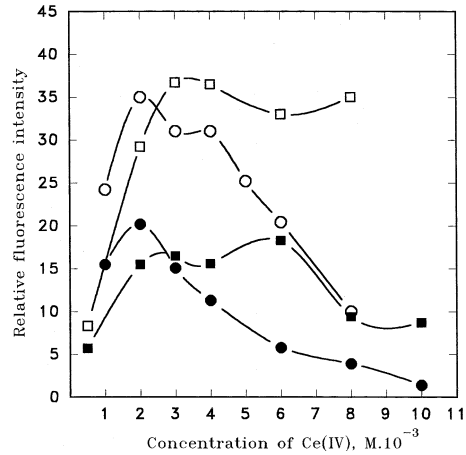

Fig. 1. Effect of Ce(IV) concentration on the fluorescence intensity induced due to oxidation of 250 (■), 500 (□) ng ml^{-1} erythromycin ethyl succinate and 250 (●), 500 (○) ng ml^{-1} azithromycin.

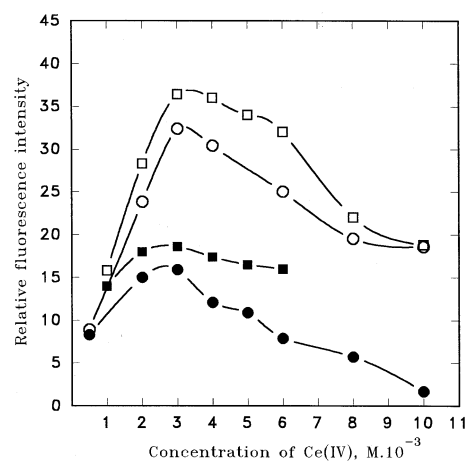

Fig. 2. Effect of Ce(IV) concentration on the fluorescence intensity induced due to oxidation of 500 (●), 1000 (○) ng ml^{-1} clarithromycin and 500 (■), 1000 (□) ng ml^{-1} roxithromycin.

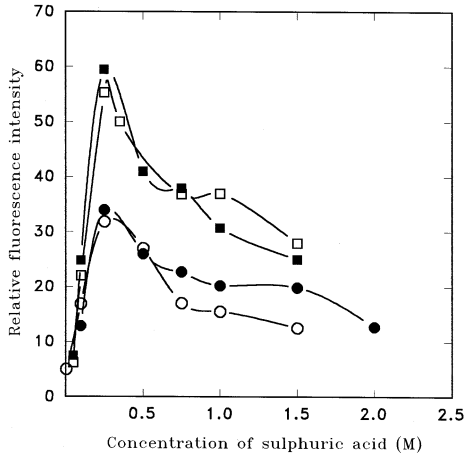

Fig. 3. Effect of sulphuric acid concentration on the fluorescence intensity induced due to oxidation of 250 ng ml^{-1} (○) erythromycin ethyl succinate, (●) azithromycin and 1000 ng ml^{-1} (□) roxithromycin, (■) clarithromycin.

and 2). By applying different volumes of the same concentration, a volume of 1 ml in a total volume of 10 ml was found to be quite enough for maximum fluorescence intensity.

3.1.2. Effect of acid conentration

Different acids as HNO_3, HCl and H_2SO_4 were tested to determine the most suitable for optimum reaction development. Either hydrochloric or sulphuric acid could be used as the fluorescence of Ce(III) is high, however sulphuric acid was selected because blank reading was less than in hydrochloric acid medium. Nitric acid could not be used due to the inhibitory effect of nitrate ions on the fluorescence of Ce(III) [33]. The effect of sulphuric acid concentration on the sensitivity of the method was studied. As shown in Fig. 3, relative fluorescence intensity increases when using 0.2–0.25 M sulphuric acid as a solvent for ammonium ceric sulphate, then decreased upon using higher concentrations. Therefore, 0.25 M sulphuric acid was adopted for this method.

3.1.3. Effect of heating temperature and time

The influence of different heating temperature and incubation time were studied using a thermostatic water bath. Accordingly, best temperature was found to be 80 °C. However complete oxidation was attained in a period of 60 min for erythromycin and azithromycin dihydrate, 45 min for clarithromycin and 30 min for roxithromycin and hence, maximum Ce(III) concentration induced (Fig. 4).

3.1.4. Effect of diluting solvents

Dilution effect with different solvents on the relative fluorescence intensity revealed that best solvents were water, MeOH, and EtOH as they were all more or less comparable to each other. However water was the solvent of choice for economic and environmental safety purposes. Other solvents as DMSO and acetonitrile showed a distinct sharp decrease in the fluorescence intensity. The induced fluorescence intensity was found stable for more than 3 h.

3.1.5. Effect of other oxidants

It is well known that some oxidized drugs show excitation and emission maximum in the same spectral region where Ce(III) fluoresces as trimeprazine. In such cases an additional enhancement of the fluorescence readings is achieved [32]. Therefore oxidation of the studied macrolides by other oxidizing agents as hydrogen peroxide, periodate, have been performed and the oxidation products were found not fluorescent. This con-

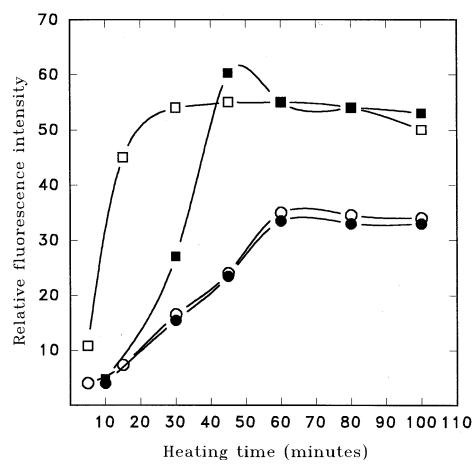

Fig. 4. Effect of heating time at 80 °C on the fluorescence intensity induced by oxidation of 250 ng ml^{-1}: erythromycin ethyl succinate (●), azithromycin (○) and 1000 ng ml^{-1} clarithromycin (■), roxithromycin (□).

Table 1
Statistical parameters for the proposed method of analysis of macrolide antibiotics

Macrolide antibiotics	Linear range (ng ml^{-1})	Least square equation ($y = a + bc$)[a]		Corr. coeff. $\pm$ S.D.[b]	RSD %	LOD[c] (ng ml^{-1})	LOQ[c] (ng ml^{-1})
		Intercept (a) $\pm$ S.D.[b]	Slope (b) $\pm$ S.D.[b]				
Erythromycin	42.6–425.7	-0.46 ± 0.64	$0.16 \pm 3.16 \times 10^{-3}$	$0.9993 \pm 5.77 \times 10^{-4}$	0.058	12.11	40.35
Azithromycin dihydrate[d]	47.7–477	-1.84 ± 0.55	$0.14 \pm 1.66 \times 10^{-3}$	$0.9952 \pm 1.9 \times 10^{-3}$	0.19	11.62	38.74
Clarithromycin	50–1200	-0.32 ± 0.2	$0.06 \pm 4.49 \times 10^{-4}$	$0.9994 \pm 1.38 \times 10^{-4}$	0.014	9.88	32.93
Roxithromycin	100–1200	0.42 ± 0.03	$0.05 \pm 9.8 \times 10^{-4}$	$0.9998 \pm 1.6 \times 10^{-4}$	0.016	17.5	58.33

[a] y = absorbance, c = concentration in ng ml^{-1}.
[b] Average of three determinations.
[c] Ref. [34].
[d] Concentration is calculated as azithromycin.

Table 2
Assay repeatability

Drug	Claimed conc. (ng ml^{-1})	% Assay[a]						Mean ± S.D.[b]	% RSD
		1	2	3	4	5	6		
Erythromycin	400	98.4	99.1	97.8	98.3	99.4	98.1	98.5 ± 0.61	0.62
Azithromycin	450	98.8	98.5	100.5	100.0	98.3	99.3	99.2 ± 0.87	0.88
Clarithromycin	1000	100.4	98.7	98.2	97.1	97.0	98.9	98.4 ± 1.27	1.29
Roxithromycin	1000	98.7	99.7	101.6	98.5	99.5	98.9	99.5 ± 1.14	1.15

[a] Results are compared with that of standard calibration curve.
[b] Average of three determinations.

firms that the fluorescence induced after oxidation of macrolides by Ce(IV) is not attributed to their oxidation products but it is mainly due to the formation of Ce(III).

3.2. Validation of the proposed method

3.2.1. Linearity, detection and quantitation limit

Using the optimal reaction conditions, Beer's law was obeyed. Linear range and regression equation using the least square method listed in Table 1 indicate the higher sensitivity of the proposed method comparing to some reported spectrophotometric [2,3,5–7,10–12], spectrofluorimetric [8,9] and even HPLC method [15]. Detection limit (LOD) and quantitation limit (LOQ) [34] for all the analytes were calculated as follows: DL or QL = kS.D.$_a$/b where $k = 3$ for DL and 10 for QL, S.D.$_a$ is the standard deviation of intercept and b is the slope. Results in Table 1 indicate that clarithromycin showed the best detection limit.

3.2.2. Repeatability

The mean of the relative fluorescence intensity of six separate samples solution of the studied macrolide of the same batch number gave a relative standard deviation of 0.62–1.29% (Table 2). This level of precision is suitable for the routine quality control analysis of pharmaceutical dosage forms.

3.2.3. Interference studies

In order to access the possible analytical applications of the proposed method, the effect of some common excipients used in pharmaceutical preparations were studied by analyzing sample solutions containing a fixed amount of roxithromycin (5.9×10^{-6} M) spiked with various excess amount of each excipient under the same reaction conditions (Table 3). Since sample solution of roxithromycin and each tested additive was prepared first in absolute ethanol then diluted to the desired concentration with distilled water, some common excipients as starch and gelatin could not be tested due to insolubility in absolute ethanol and hence could not interfere. The recovery results show that no serious interference occurred from the classical additives tested. The tolerance ratio of each foreign compound was taken as the largest amount yielding an error of not less than ± 4%. Some tested excipients which are reducing substances as glucose, sucrose, and lactose were tolerated in 100-fold excess m/m over roxithromycin. While citric acid was tolerated in 5-fold excess.

Table 3
Tolerance of the proposed method to interference

Additives	Tolerance molar ratio (m/m)	% Recovery ($n = 3$)
Sucrose	100	99.8
Lactose	90	100.1
Glucose	80	100.1
Citric acid	5	98.7
Propylene glycol	1	98.0

5.9×10^{-6} M roxythromycin tested as a representative example of the studied macrolide antibiotics.

Table 4
Assay of macrolide antibiotics in pharmaceutical dosage forms by the proposed and reported method

Sample[a]	Nominal value (mg)	Found[b] by proposed method (% ± S.D.[c])	Found[b] by reported[d] method (% ± S.D.[c])	Recovery[e] by proposed method (% ± S.D.[c])
Erythromycin tablets	250/tablet	98.3 ± 0.8 $F = 1.44$ $t = 2.33$	98.8 ± 0.96	98.6 ± 0.82
Erythrocin granules	200/teasponful	99.3 ± 0.8 – –	–	97.7 ± 0.9
Zithromax capsules	250/capsule	100.5 ± 0.3 – –	–	99.2 ± 0.6
Klacid tablets	250/tablet	99.6 ± 0.8 $F = 1.39$ $t = 1.28$	100.2 ± 0.7	100.7 ± 1.6
Rulid tablets	50/tablet	100.8 ± 0.3 $F = 1.89$ $t = 2.26$	100.3 ± 0.4	100.9 ± 0.8

[a] See experimental part for suppliers and preparations.
[b] Theoretical value at 95% confidence limit and $n = 5$ for F is 6.39 and t is 2.78.
[c] Average of five determinations.
[d] Ref. [4] for erythromycin and Ref. [12] for both clarithromycin and roxithromycin.
[e] Recovery of 1.0 mg erythromycin, 1.0 mg azithromycin, 2.0 mg clarithromycin, and 2.0 mg roxithromycin (added to its corresponding dosage forms) by standard addition method [35].

3.2.4. Accuracy and recovery

Applying the suggested spectrofluorimetric procedure for the analysis of commercially available dosage forms validated the accuracy of the proposed method. Table 4 shows mean percentage recoveries of 98.3–100.8% (± 0.3–0.8 S.D.) of the labelled amount. This indicates an excellent concordance between experimental and nominal values. The performance of the current method was judged by comparing with other reported methods. According to the variance ratio test (F-test), and t-test, the calculated values of F and t listed in Table 4 indicate that there is no significant difference between the proposed and reported method [4,12] with respect to precision and accuracy. To confirm the accuracy of the method, recovery studies were performed by using the standard addition method [35]. This depends upon the addition of a fixed known quantity of the standard macrolide antibiotic being determined to known quantities of the corresponding pharmaceutical sample antibiotic, then analyze the resulting solutions by the proposed method. The relative fluorescence intensity after each addition was plotted against concentration of the sample. Extrapolation of the resulting line intercepts the negative horizontal axis at the concentration of the added standard antibiotic. Results obtained indicate good recoveries (97.7–100.9% ± 0.6–1.6) and confirm the absence of interference due to common ingredients and excepients and hence, accuracy of the proposed method (Table 4).

3.2.5. Specificity

According to the results obtained by both recovery experiment and interfence study, the proposed method is able to access the analyte in the presence of common excepients and hence, it can be considered specific.

Table 5
Influence of small variation in the assay condition of the proposed method on the method suitability test parameters and sensitivity

Variation	λ_{ex} (nm)	λ_{em} (nm)	Assay % ($n = 3$)			
			Erythromycin	Azithromycin	Clarithromycin	Roxithromycin
No variation	255	348	99.3	99.0	99.4	99.8
Ce (IV) weight (g)						
For AZ						
1.26	258	348	–	98.7	–	–
1.28	258	348	–	99.2	–	–
For other drugs						
1.89	256	346	99.1	–	99.0	99.4
1.91	255	346	99.2	–	99.1	99.5
H_2SO_4 volume (ml)						
1.34	256	348	98.6	98.4	98.6	99.3
1.38	255	347	98.6	98.3	98.6	99.4
Heating temp. (°C)						
77	255	348	98.9	99.1	99.4	99.1
83	255	348	99.2	99.3	99.3	99.6
Incubaiom time (min.)						
For ER and AZ						
63	255	346	98.7	99.6	–	–
67	254	345	98.4	99.0	–	–
For ClA						
43	256	345	–	–	98.4	–
47	255	348	–	–	98.6	–
For ROX						
35	255	347	–	–	–	99.0
45	255	348	–	–	–	99.3

3.2.6. Robustness [36]

It was examined by evaluating the influence of small variation of five method variables including: Ce(IV), sulphuric acid concentrations, heating temperature and times on the method suitability and sensitivity. None of these variables significantly affected the relative fluorescence intensity of the induced Ce(III) (Table 5). This provides an indication of the reliability of the proposed method during normal usage and so it could be considered robust [36].

3.2.7. Ruggedness [36]

It was tested by applying the proposed method to the assay of studied macrolides using the same operational conditions but using different lab and different elapsed time. Results obtained due to lab-to-lab and day-to-day variation were found reproducible as RSD did not exceed 2%.

4. Conclusion

The present work is the first to describe a fully validated spectrofluorimetric procedure for the assay of macrolide antibiotics without interference from common excepients. Hence, it can be recommended for the routine quality control of these antibiotics in their pharmaceutical dosage forms. Another advantage, is that, compared to the existing spectrofluorimetric method of erythromycin it is 100 times more sensitive. From the economical point of view, the proposed method is simple, rapid and inexpensive so it is a good alternative to

reported antimicrobial methods and to high cost HPLC with an equivalent sensitivity. A part from that, the possibility of using the proposed method as a stability indicating assay is not currently our main aim, however this work will be our goal of interest in the near future.

References

[1] J.N. Delgado, W.A. Remers, Textbook of Organic Medicinal and Pharmaceutical Chemistry, 10th ed., Lippincott-Raven, Philadelphia, PA, 1998, pp. 307–312.
[2] R.V. Smith, R.G. Harris, E. Sanchez, D.D. Manees, Microchem. J. 22 (1997) 168–175.
[3] El.-S.A. Ibrahim, Y.A. Beltagy, A.S. Issa, Pharmazie 27 (10) (1972) 651–652.
[4] N.D. Danielson, J.A. Holeman, D.C. Bristol, D.H. Kirzner, J. Pharm. Biomed. Anal. 11 (2) (1993) 121–130.
[5] P.A. Gallagher, N.D. Daneilson, Talanta 42 (1995) 1425–1432.
[6] A.S. Issa, M.A. Abdel-Salam, H.M.G. Daabees, N.S. Boni, Alex. J. Pharm. Sci. 4 (1) (1990) 7–11.
[7] A.S. Amin, Y.M. Issa, J. Pharm. Biomed. Anal. 14 (11) (1996) 1625–1629.
[8] A. Sanz, V. Tomas, C. Martinez-Lozano, T. Perez-Ruiz, Analyst 118 (1993) 567–571.
[9] K.Y. Tserng, J.G. Wagner, Anal. Chem. 48 (2) (1976) 348–353.
[10] C.S.P. Sastry, K.R. Rao, D.S. Prassad, Microchim. Acta 122 (1–2) (1996) 53–60.
[11] C.S.P. Sastry, S.G. Rao, K. Rama Strinivas, Indian Drug 35 (9) (1998) 594–596.
[12] I.I. Hamdan, A.M. Mishal, Saudi Pharm. J. 8 (4) (2000) 191–197.
[13] L. Kanfer, M.F. Skinner, R.B. Walker, J. Chromatogr. A 812 (1–2) (1998) 255–286.
[14] K. Tsuji, M.P. Kane, J. Pharm. Sci. 71 (10) (1982) 1160–1164.
[15] T. Geria, W.-H. Hong, R.E. Daly, J. Chromatogr. 396 (1987) 191–198.
[16] The United States Pharmacopoeia, The National Formulary, USP 24, NF 19, USP Convention Inc., 12601 Twinbrook Parkway, Rockville MD, 2000, pp. 185–187, 424–425, 663–676.
[17] British Pharmacopoeia, vol. II, London, The Stationery Office, 1998, pp. 1663–1668.
[18] J. Macek, P. Ptacek, J. Klima, J. Chromatogr. Biomed. Sci. 723 (1–2) (1999) 233–238.
[19] P.O. Erah, D.A. Barrett, P.N. Shaw, J. Chromatogr. B Biomed. Appl. 682 (1) (1996) 73–78.
[20] E. Dreassi, P. Corti, F. Bezzini, S. Furlanetto, Analyst 125 (6) (2000) 1077–1081.
[21] C. Taninaka, H. Ohtani, E. Hanada, H. Kotaki, H. Sato, T. Iga, J. Chromatogr. B Biomed. Sci. Appl. 738 (2) (2000) 405–411.
[22] D.A. Raines, A. Yusuf, M.H. Jabak, W.S. Ahmed, Z.A. karcioglu, A. El-Yazigi, Ther. Drug Monit. 20 (6) (1998) 680–684.
[23] F. kees, S. Spangler, M. Wellenhofer, J. Chromatogr. A 812 (1–2) (1998) 287–293.
[24] H. Toreson, B.M. Eriksson, J. Chromaogr. B Biomed. Appl. 673 (1) (1995) 81–89.
[25] M. Hedenmo, B.M. Eriksson, J. Chromatogr. 692 (1–2) (1995) 161–166.
[26] J. Sastre Toranto, H.J. Guchelaar, J. Chromatogr. Biomed. Sci. 11/720 (1–2) (1998) 89–97.
[27] K. Tsuji, J. Chromatogr. 158 (1987) 337–348.
[28] J.A. Bernabeu, M.A. Camacho, M.E. Gil Alegre, V. Ruz, Al. Torres-Suarez, J. Pharm. Biomed. Anal. 21 (2) (1999) 347–353.
[29] P.K. Markakis, J. AOAC Int. 79 (6) (1996) 1263–1268.
[30] A. Lliopoulou, R.N. Thin, P. Turner, Br. J. Vener. Dis. 57 (4) (1981) 263–267.
[31] C.C. Lai, P.L. Tsai, C. Yu, G.R. Her, Rapid Commun. Mass. Spectrom. 14 (6) (2000) 468–475.
[32] T. Perez-Ruitz, C. Martinez-Lozano, V. Tomas, C. Sidrach de Cardona, Talanta 40 (9) (1993) 1361–1365.
[33] P. Cukor, R.P. Weberling, Anal. Chim. Acta 41 (1968) 401.
[34] J.N. Miller, Analyst 116 (1991) 3–13.
[35] G.W. Ewing, Instrumental Methods of Chemical Analysis, 5th ed., Lippincott-Raven, Philadelphia, PA, 1995, pp. 484–486.
[36] The United States Pharmacopoeia, The National Formularly, USP 24, NF 19, USP Convention Inc., 12601 Twinbrook Parkway, Rockville MD, 2000, pp. 2151–2152.