Acta Pharm. 56 (2006) 431–440

Original research paper

Spectrofluorimetric determination of ciclopirox olamine *via* ternary complex with Tb(III) and EDTA

MOHAMED IBRAHIM WALASH*
MOHAMED SALEM RIZK
MANAL IBRAHIM EID
MONA EL SAYED FATHY

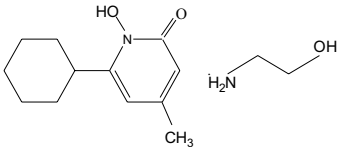
Department of Analytical Chemistry
Faculty of Pharmacy
University of Mansoura
Mansoura 35516, Egypt

A highly sensitive and selective spectrofluorimetric method was developed for the determination of ciclopirox olamine in raw material and in dosage forms. The proposed method is based on the formation of a ternary complex with Tb(III) in the presence of ethylenediaminetetraacetic acid. It was found that this complex manifests intense fluorescence at λ_{em} 489 and 545 nm with excitation at 295 nm. Different experimental parameters affecting the fluorescence intensity of the complex were carefully studied and incorporated into the procedure. Under the described conditions, the method is applicable over the concentration range 30–150 and 10–70 ng mL^{-1} with minimum detectability of 6.7 and 0.9 ng mL^{-1} at λ_{em} 489 and 545 nm, respectively. The mean percentage recovery at λ_{em} 489 and λ_{em} 545 nm ranged between 98.7 and 100.2 for the pure substance, solution, and cream. Relative error of 0.1–0.4% and *RDS* of up to 0.9% were estimated at λ_{em} 489 and 545 nm. A proposal of the reaction pathway is given.

Keywords: ciclopirox olamine, Tb(III), EDTA, spectrofluorimetry, dosage forms

Accepted May 18, 2006

Ciclopirox olamine [(6-cyclohexyl-1-hydroxy-4-methyl-2(1H)-pyridone)-2-amino ethanol (1:1)] is a wide-spectrum antifungal agent:



It inhibits most *Candida*, *Epidermophyton*, *Microsporum* and *Trichophyton* species and is also active against *Malassezia* (1). It also shows some antibacterial activity. UV absorption spectrophotometry is used for ciclopirox olamine determination either in its pure form,

* Correspondence, e-mail: manal_eid@yahoo.com

cream, or topical suspension (2). Other reported methods include high-performance liquid chromatography (3–6), thin layer chromatography (7) and polarography (8).

Continuing the investigation in our laboratory on the utility of lanthanide ions for the formation of fluorescent derivatives (9–12), this article is devoted to developing a simple spectrofluorimetric method for the analysis of ciclopirox olamine based on the reaction of the drug with Tb(III) and EDTA. Review of the literature revealed that no spectrofluorimetric methods have been reported for assaying the studied drug substance or drug formulations.

EXPERIMENTAL

Apparatus

A RF-1501 Shimadzu spectrofluorophotometer (Japan) with a xenon lamp was used with the excitation and emission slits set at 5 mm. A 1-cm quartz cell was used for all measurements.

Materials and reagents

A pure drug sample of ciclopirox olamine was kindly supplied by Egyptian International Pharmaceutical Industries Co. (Egypt) and its purity was established by applying the USP method (2). Batrafen solution is the product of Global Napi Pharmaceuticals (Egypt); each 1 mL contains 10 mg of ciclopirox olamine. Batrafen cream is the product of Hoechst Orient S. A. E. (Egypt); each 1 g contains 10 mg of ciclopirox olamine. All chemicals used were of analytical reagent grade.

Borate buffer (0.2 mol L^{-1}), covering the pH range 5–11 (2), was used. Terbium(III) chloride was purchased from Sigma Chemical Company (USA). Disodium salt of EDTA was obtained from Merck (Germany).

An aqueous solution of terbium chloride (1×10^{-3} mol L^{-1}) and 1.5×10^{-3} mol L^{-1} aqueous solution of EDTA salt were prepared and used throughout the study.

Methanol 96% (Merck) and doubly distilled water were used.

Stock solution

About 100 mg of ciclopirox olamine, accurately weighed, was transfered to a 100-mL volumetric flask, dissolved in about 80 mL of methanol, diluted with methanol to volume, and mixed. Serial dilutions with methanol were prepared (0.1 to 1.5 μg mL^{-1}).

Procedure

Transfer 1.0 mL of final ciclopirox olamine solution into a series of 10-mL volumetric flasks. Add 2 ml of borate buffer (pH 10) to each flask followed by 2.5 mL of 1×10^{-3} mol L^{-1} of $TbCl_3$ solution, and 1.5 mL of 1.5×10^{-3} mol L^{-1} of EDTA solution (final concentration of 2.5×10^{-5} and 2.3×10^{-5} mol L^{-1} for $TbCl_3$ and EDTA, respectively). Make up the volume to the mark with water. Mix well and measure the relative fluorescence intensity

(RFI) of the solution at λ_{em} 489 and 545 nm using 295 nm as the excitation wavelength. A blank experiment is prepared simultaneously omitting the drug.

Determination of ciclopirox olamine in solution. – Transfer an accurately measured volume of Batrafen solution, equivalent to about 10 mg of ciclopirox olamine, to a 50-mL volumetric flask, dilute to volume with methanol and mix well; successively dilute to obtain final concentrations ranging from 0.1 to 1.5 μg mL^{-1}; proceed as described under preparation of the calibration curve.

Determination of ciclopirox olamine in cream. – Transfer an accurately weighed quantity of Batrafen cream, equivalent to about 10 mg of ciclopirox olamine, to a 50-mL volumetric flask, add 25 mL of methanol, and shake mechanically for about 10 minutes. Dilute with methanol to volume, mix, centrifuge, and dilute the supernatant liquid with methanol to get final concentrations ranging from 0.1 to 1.5 μg mL^{-1}; proceed as described above.

RESULTS AND DISCUSSION

The drug-Tb-EDTA ternary complex was found to exhibit intense fluorescence at 489 and 545 nm after excitation at 295 nm (Fig. 1). The fluorophore was formed instantaneously and remained stable for at least 90 minutes. However, the drug-Tb binary com-

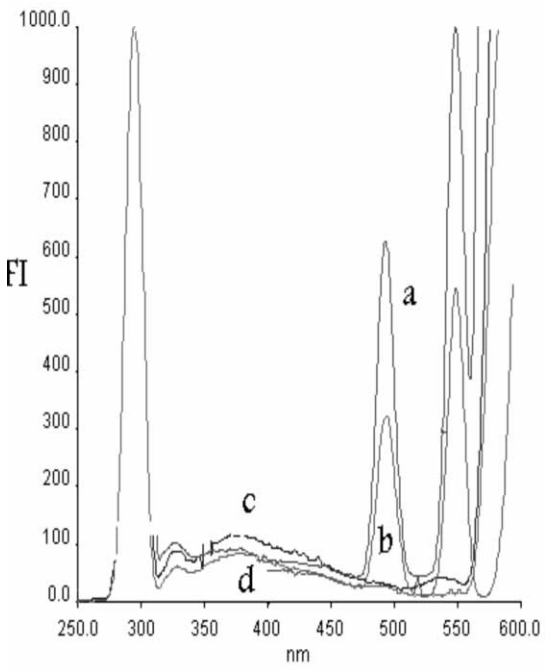


Fig. 1. Fluorescence spectra of ciclopirox olamine complexes: a) ciclopirox (70 ng mL^{-1})–Tb(III) (2.5×10^{-5} mol L^{-1})–EDTA (2.3×10^{-5} mol L^{-1}); b) ciclopirox (700 ng mL^{-1})–Tb(III) (2.3×10^{-5} mol L^{-1}); c) ciclopirox (700 ng mL^{-1})–EDTA (2.3×10^{-5} mol L^{-1}); d) Tb(III) (2.3×10^{-5} mol L^{-1})–EDTA.

plex was found to exhibit poor fluorescence compared to the ternary complex, as shown in Fig. 1.

Ciclopirox olamine is a hydroxypyridone antifungal that is structurally unrelated to the common imidazole derivatives or other antifungals (1). Intense fluorescence of the produced ternary complex at 489 and 545 nm allows the determination of ciclopirox olamine in combination with oral antifungals such as terbinafine, itraconazole and griseofluvin with a good selectivity.

Optimizing the experimental conditions

The effect of pH, EDTA concentration, Tb(III) concentration and reaction time on the fluorescence intensity of the ternary complex formed were studied. The fluorescence intensity of the ternary complex was investigated over the pH range 5–11 (2). The maximum fluorescence intensity was achieved at pH 10 ± 0.5. The effect of Tb(III) concentration on the fluorescence intensity of the ternary complex was also studied. It was observed that the final concentration of terbium chloride and EDTA of 2.5×10^{-5} and 2.3×10^{-5} mol L^{-1} assured the maximum fluorescence intensity. The fluorophore was formed instantaneously and remained stable at room temperature (25 °C) for at least 90 minutes, as shown in Fig. 2.

To optimize the assay parameters, one parameter was changed whereas the others were kept unchanged, and the recovery was calculated each time. It was found that none of examined variables significantly affected the performance of the method; the recovery values ranged between 98.4 ± 0.8 and 100.3 ± 0.4% (Table I). This provides an indication of the reliability of the proposed method during its routine application.

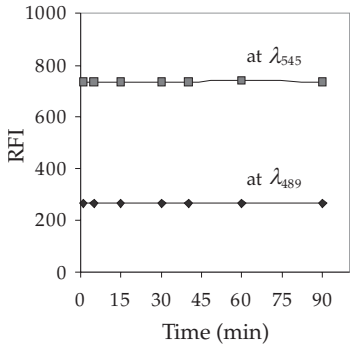


Fig. 2. Effect of time on the fluorescence intensity of the formed ciclopirox olamine (60 ng mL^{-1}) – Tb^{3+}–EDTA ternary complex.

The results for the authentic drug sample obtained by the proposed methods were compared with those obtained by the official potentiometric method (2). The calculated *t*- and *F*-values did not exceed the theoretical values indicating that there is no significant difference between the proposed and official methods (Table II).

Table I. Optimization of assay conditions[a]

Parameter	Recovery ($\overline{X} \pm SD$) (%)[b]	
	at λ_{489}	at λ_{545}
Tb^{3+} concentration (mol L^{-1})		
2.5×10^{-5}	99.5 ± 0.1	100.3 ± 0.4
3.5×10^{-5}	98.9 ± 0.5	99.7 ± 0.8
EDTA concentration (mol L^{-1})		
1.8×10^{-5}	98.4 ± 0.8	99.91 ± 0.63
2.8×10^{-5}	99.4 ± 0.2	99.62 ± 0.83
pH of borate buffer (0.2 mol L^{-1})		
9.25	98.9 ± 0.5	100.0 ± 0.6
10.25	99.7 ± 0.04	100.1 ± 0.5

[a] 70 ng mL^{-1} ciclopinox olamine; drug: Tb(III): EDTA molar ratio 3:1:1.
[b] Each result is the average of three results.

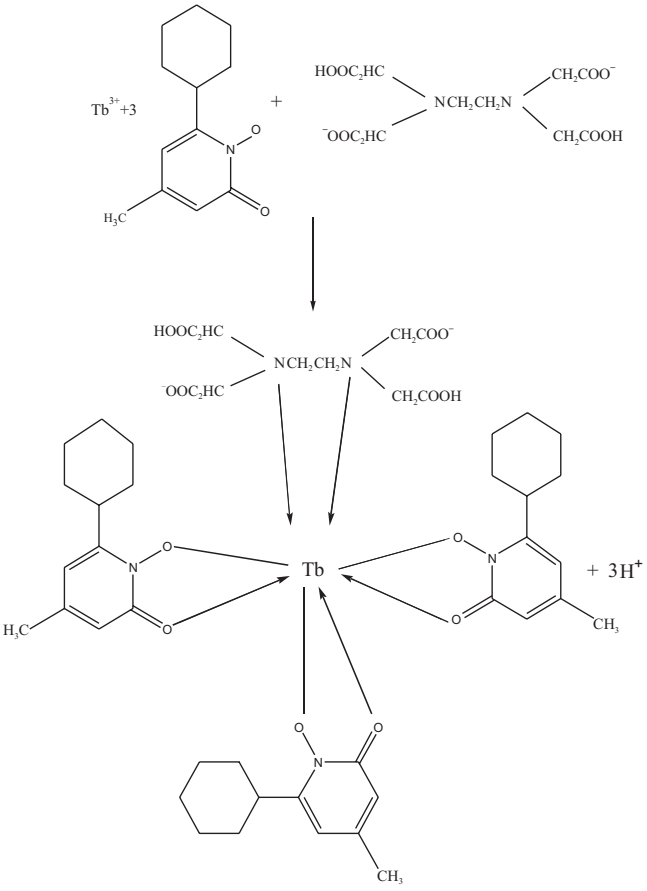
Table II. Spectrofluorimetric determination of ciclopirox olamine in its pure form

Proposed method						Official method (2)		
at λ_{489}			at λ_{545}					
Added (ng mL^{-1})	Found (ng mL^{-1})	Recovery (%)	Added (ng mL^{-1})	Found (ng mL^{-1})	Recovery (%)	Added (mg mL^{-1})	Found (mg mL^{-1})	Recovery (%)
30.0	30.6	101.9	10.0	9.8	98.2	48.0	48.8	101.1
40.0	40.2	100.5	20.0	19.9	99.7	80.0	78.8	98.4
60.0	59.8	99.6	30.0	30.3	100.8	112.0	112.2	100.2
70.0	70.2	100.2	40.0	40.0	100.0	144.0	144.2	100.1
80.0	79.1	98.9	50.0	50.0	100.0			
100.0	99.3	99.3	60.0	60.5	100.8			
140.0	140.8	100.6	70.0	69.6	99.4			
150.0	150.0	100.0						
$\overline{X} \pm SD$		100.1 ± 0.9			99.8 ± 0.9			100.0 ± 1.1
F-value		1.37 (4.35)			1.49 (4.76)			
t-value		0.32 (2.22)			0.21 (2.26)			
RSD (%)		0.9			0.9			1.1
LOD		6.7 ng mL^{-1}			0.9 ng mL^{-1}			26.8 mg mL^{-1}
LOQ		30 ng mL^{-1}			10 ng mL^{-1}			48 mg mL^{-1}

[a] Each result is the average of three separate determinations.
The figures in brackets are the tabulated values of *F* and *t* at $p = 0.05$. Comparison of recovery values between each variant and the official method is done.

Proposed mechanism of the reaction

The stoichiometry of the reaction was determined by the limiting logarithmic method (14). The fluorescence intensity of the reaction product was alternatively measured in the presence of excess of Tb(III) and ciclopirox olamine. A plot of log RFI *vs.* log [Tb(III)] and log [ciclopirox olamine] gave straight lines, the values of the slopes being 0.39 and 1.09, respectively (Fig. 3). Hence it is concluded that the reaction proceeds in the ratio of 1:3 [Tb(III):ciclopirox olamine]. As the drug belongs to hydroxamic acid derivatives, which are known to be bidentate ligands, and since Tb(III) has a coordination number of 6 or 8, it is suggested that it combines with three molecules of the drug *via* 3 covalent bonds and 3 dative bonds in addition to 2 dative bonds with EDTA. The proposed mechanism of the reaction of ciclopirox olamine with Tb(III) in the presence of EDTA is illustrated in Scheme 1.



Scheme 1.

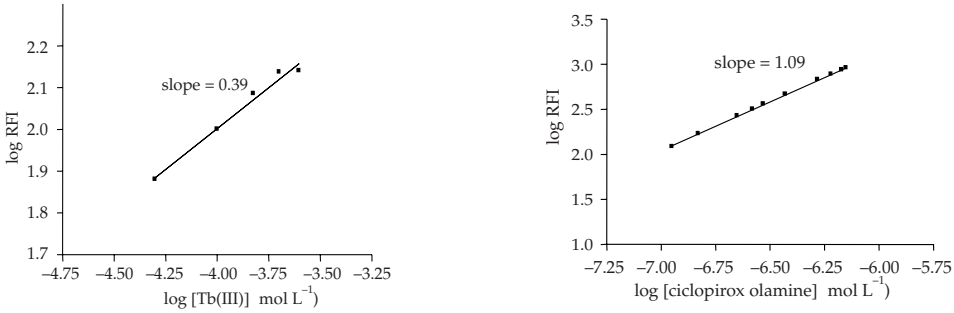


Fig. 3. Limiting logarithmic plots for the molar ratio.

Figures of merit

After optimizing the conditions, it was found that the relation RFI and the final concentration of the drug was linear over the range 30–150 ng mL^{-1} with recovery of 100.1 ± 0.9% for λ_{em} 489 nm and 10–70 ng mL^{-1} with recovery of 99.8 ± 0.9% for λ_{em} 545 nm. Linear regression analysis of the data gave the following equations:

$$\text{at } \lambda_{489}\text{: RFI} = -30.63 + 4.990\,\gamma \quad (R = 0.9999)$$

$$\text{at } \lambda_{545}\text{: RFI} = -6.54 + 12.247\,\gamma \quad (R = 0.9999)$$

γ is the final concentration (ng mL^{-1}).

Table III. Accuracy, precision and selectivity data for pure ciclopirox olamine using the proposed method

	at λ_{489}				at λ_{545}			
Parameter	Concentration (ng mL^{-1})	$\overline{X}$ ± SD (%)[a]	*RSD* (%)	e_r (%)	Concentration (ng mL^{-1})	$\overline{X}$ ± SD (%)[a]	*RSD* (%)	e_r (%)
Intra-day	80.0	99.9 ± 0.6	0.6	0.3	40.0	100.4 ± 0.5	0.5	0.3
	120.0	100.0 ± 0.3	0.3	0.1	60.0	100.8 ± 0.7	0.7	0.4
	150.0	100.3 ± 0.5	0.5	0.3	70.0	99.2 ± 0.5	0.5	0.3
Inter-day	80.0	100.2 ± 0.6	0.6	0.3	40.0	99.8 ± 0.5	0.5	0.3
	120.0	99.6 ± 0.7	0.7	0.4	60.0	100.2 ± 0.3	0.3	0.2
	150.0	100.1 ± 0.5	0.5	0.3	70.0	99.2 ± 0.5	0.5	0.3
Selectivity[b]	40.0[c]	100.4 ± 0.1	0.1	0.1	40.0[c]	100.2 ± 0.3	0.3	0.2
	60.0[d]	99.3 ± 1.1	1.1	0.6	60.0[d]	100.3 ± 1.1	1.1	0.6
	70.0[e]	99.5 ± 0.9	0.9	0.5	70.0[e]	99.3 ± 0.2	0.2	0.1

[a] Mean ± SD (n = 3).
[b] Recovery in the presence of common excipients (100 mg ciclopirox : 1 g excipient): [d] lanoline, [e] polyethylene glycol, [f] Tween 40.

Analysis of the data for the authentic sample gave low values of standard deviations of the residuals ($s_{y/x}$) 3.11 and 4.18, of slope (s_b) 0.03 and 0.08, and of intercept (s_a) 0.89, and 1.48 for emission at 489 and 545 nm, respectively.

The limit of quantitation (*LOQ*), which is the lowest amount of analyte in a sample that can be determined with acceptable precision and accuracy under the stated experimental conditions (2), was 30 and 10 ng mL^{-1} for 489 and 545 nm, respectively. The limit of detection (*LOD*), defined as the analyte concentration giving a signal equal to the blank signal plus two standard deviations of the blank (15), was 6.7 and 0.9 ng mL^{-1} for 489 and 545 nm, respectively.

The precision and accuracy of the proposed method were calculated using standard solutions containing three different concentrations of ciclopirox olamine (three replicates). The mean results obtained are summarized in Table III. Low relative standard deviation of up to 0.9% for both λ_{em} can be considered adequate for the quality control analysis of pharmaceutical preparations. For the evaluation of accuracy, recovery was calculated using the calibration curve; accuracy was also expressed as the mean relative

Table IV. Spectrofluorimetric determination of ciclopirox olamine in dosage forms

Pharmaceutical preparation	Proposed method					Official method (2)		
	at λ_{489}			at λ_{545}				
	Added (ng mL^{-1})	Found (ng mL^{-1})[a]	Recovery (%)	Found (ng mL^{-1})[a]	Recovery (%)	Added (ng mL^{-1})	Found (ng mL^{-1})[a]	Recovery (%)
Batrafen solution	40.0	40.1	100.3	39.4	98.4	24.0	24.0	99.5
(10 mg of ciclopirox	60.0	59.1	98.5	59.0	98.3	40.0	39.4	98.5
olamine per mL)	70.0	69.7	99.6	70.1	100.1	56.0	56.6	101.0
						72.0	71.4	99.2
$\overline{X}$ ± SD			99.5 ± 0.9		99.0 ± 1.0			99.7 ± 1.1
F-value			1.4 (19.16)		1.1 (5.79)			
t-value			0.3 (2.571)		0.9 (2.365)			
Batrafen cream	40.0	40.1	100.3	40.2	100.5	24.0	24.0	100.2
(10 mg ciclopirox	60.0	59.0	98.3	60.6	101.0	32.0	31.6	98.8
olamine per g)	70.0	69.0	98.6	69.4	99.1	40.0	39.4	98.5
						56.0	56.6	101.1
						64.0	63.7	99.5
						72.0	71.3	99.0
$\overline{X}$ ± SD			99.1 ± 1.1		100.2 ± 1.0			99.5 ± 1.0
F-value			1.2 (9.55)		1.0 (19.3)			
t-value			0.8 (2.571)		1.3 (2.365)			

[a] Each result is the average of three separate determinations.
The figures in brackets are the calculated rules of *F* and *t* at $p = 0.05$. Comparison of recovery values between each variant of the proposed method and the official method.

error (e_r = 0.1–0.4%). From the results presented in Table III, it can be concluded that both wavelengths examined are suitable for quantitative determination of ciclopirox olamine; markedly higher sensitivity was achived at 545 nm. Selectivity was confirmed by adequate recovery obtained in the presence of multifold excess of lanoline, polyethylene glycol and Tween 40 (Table III).

The proposed method was successfully applied to the assay of ciclopirox olamine in different dosage forms, including cream and solution. The results shown in Table IV are in good agreement with those obtained with the official method (2). The USP recommended spectrophotometric method for the determination of ciclopirox olamine in cream and solution is time consuming and less sensitive than the proposed spectrofluorimetric method. The concentration range of the official method is in mg mL^{-1} and μg mL^{-1} for the pure substance and pharmaceuticals, respectively, while it is in ng mL^{-1} for the proposed spectrofluorimetric method. However, as shown in Table III, a potentiometric method recommended by USP for the determination of ciclopirox olamine in pure form is not selective and is less sensitive than the proposed method.

CONCLUSIONS

The proposed method has the advantages of being simple, sensitive and suitable for routine analysis in control laboratories. It could be applied to the analysis of ciclopirox olamine in different pharmaceutical dosage forms.

REFERENCES

1. Martindale, *The Complete Drug Reference*, 32nd ed., The Pharmaceutical Press, London 1999, p. 376.
2. *The US Pharmacopeia XXVII, NF 22*, The US Pharmacopeial Convention, Rockville 2004.
3. L. Galgliardi, G. Multuri, G. Cavazzutti, D. De-Oris and D. Tonelli, HPLC determination of ciclopirox, octopirox, and antidandruff preparations, *J. Liq. Chromatogr. Rel. Technol.* **21** (1998) 2365–2373.
4. K. H. Lehr and P. Damm, Quantification of ciclopirox by high-performance liquid chromatography after pre-column derivatization. An example of efficient clean-up using silica-bonded cyano phases, *J. Chromatogr. Biomed. Appl.* **40** (1985) 451–456.
5. G. Coppi and S. Silingardi, HPLC method for pharmacokinetic studies on ciclopirox olamine in rabbits after intravenous and intravaginal administrations, *Farmaco* **47** (1992) 779–786.
6. F. Belliardo, A. Bertolino, G. Brandolo and C. Lucarelli, Micro-liquid chromatography method for the determination of ciclopirox olamine after precolumn derivatization in topical formulations, *J. Chromatogr.* **553** (1991) 41–45.
7. T. Kuriki, T. Tsujiyama, R. Gurniak and N. Suzuki, Study on the thin-layer chromatography of 1-hidroxy-4-methyl-6-cyclohexyl-2-pyridine ethamolamine salt, *Bunseki Kagaku* [*Japan Analyst*] **25** (1976) 406–408, ref. *Chem. Abst.* **85** (1976) 130585k.
8. F. Ibrahim and N. El-Enany, Anodic polarographic determination of ciclopirox olamine in pure and certain pharmaceutical preparations, *J. Pharm. Biomed. Anal.* **32** (2003) 353–359.

9. M. Rizk, Y. El-Shabrawy, N. A. Zakhari, S. S. Toubar and L. A. Carreira, Spectroscopic determination of heparin sodium using Eu[III] ions as probe ions, *Spectrosc. Lett.* **28** (1995) 1235–1249.
10. M. Rizk, F. Belal, F. Ibrahim, S. M. Ahmed and N. M. El-Enany, Fluorimetric determination of oxamniquine in biological fluids, *Farmaco* **54** (1999) 47–50.
11. M. Rizk, F. Belal, F. A. Aly and N. M. El-Enany, Fluorimetric determination of nalidixic acid in formulations and biological fluids through ternary complex formation, *Anal. Lett.* **30** (1997) 1897–1908.
12. M. Rizk, F. Belal, F. Ibrahim, S. Ahmed and N. El-Enany, Spectrofluorimetric analysis of certain 4-quinolones in pharmaceuticals and biological fluids, *Pharm. Acta Helv.* **74** (2000) 371–377.
13. R. G. Alfonso Remington, *The Science and Practice of Pharmacy*, 20th ed., Lipincott Williams and Wilkins, Philadelphia 2000, pp. 135–140.
14. J. Rose, *Advanced Physicochemical Experiments*, Pitman, London 1964, p. 67.
15. J. C. Miller and J. N. Miller, *Errors in Instrumental Analysis; Regression and Correlation*, in *Statistics for Analytical Chemistry*, 3rd ed., Ellis-Horwood, New York 1993, p. 115.

S A Ž E T A K

Spektrofluorimetrijsko određivanje ciklopiroks olamina prevođenjem u ternarni kompleks s Tb(III) i EDTA

MOHAMED IBRAHIM WALASH, MOHAMED SALEM RIZK, MANAL IBRAHIM EID i MONA EL SAYED FATHY

Razvijena je vrlo osjetljiva i selektivna spektrofluorimetrijska metoda za određivanje antimikotika ciklopiroks olamina, kao čiste supstancije i u ljekovitim oblicima. Metoda se temelji na stvaranju kompleksa s Tb(III) u prisutnosti etilendiamintetraoctene kiseline. Nakon ekscitacije pri 295 nm taj kompleks intenzivno fluorescira pri λ_{em} 489 i 545 nm. Proučavani su različiti eksperimentalni parametri koji utječu na intenzitet fluorescencije kompleksa. Za opisane uvjete metoda se može primijeniti u koncentracijskom području 30–150 i 10–70 ng mL^{-1}. Minimalna koncentracija koja se može odrediti je 6,7 odnosno 0,9 ng mL^{-1} na λ_{em} 489 odnosno 545 nm. Analitički povrat pri λ_{em} 489 i λ_{em} 545 nm iznosio je 98,7–100,2% za čistu supstanciju, otopinu i kremu. Relativna pogreška metode je 0,1–0,4%, a relativna standardna devijacija 0,9%. Predložena je jednažba kemijske reakcije.

Ključne riječi: ciklopiroks olamin, Tb(III), EDTA, spektrofluorimetrija, ljekoviti oblici

Department of Analytical Chemistry, Faculty of Pharmacy, University of Mansoura, Mansoura 35516, Egypt