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ORIGINAL PAPER

Spectrofluorimetric Determination of Terbinafine Hydrochloride and Linezolid in their Dosage Forms and Human Plasma

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Abstract A highly sensitive, simple and rapid spectrofluorimetric method was developed for the determination of Terbinafine HCl (TRH) and linezolid (LNZ) in their pharmaceutical formulations. The proposed method is based on measuring the native fluorescence of the studied drugs in water at 336 nm after excitation at 275 nm for TRH and 375 nm after excitation at 254 nm for LNZ. The fluorescence–concentration plots were rectilinear over the range of 0.02–0.15 μg/mL for TRH and 0.5–5.0 μg/mL for LNZ. With lower detection limits of 3.0 and 110.0 ng/mL and a lower quantification limit of 9.0 and 320.0 ng/mL for TRH and LNZ, respectively. The method was successfully applied to the analysis of TRH in its commercial tablets, cream, gel and spray formulations and the results were in good agreement with those obtained with the official method. In addition the method was also applied to the analysis of LNZ in its capsule and I.V solution and the results were in good agreement with those obtained with the comparison method. The effect of sensitizers was studied. The method was extended to the determination of the studied drugs in spiked human plasma and the results were satisfactory.

Keywords Terbinafine HCl · Linezolid · Spectrofluorimetry · Pharmaceutical formulations · Human plasma

Introduction

Terbinafine hydrochloride (Fig.1a); (*E*)-*N*-(6,6-dimethyl-2-hepten-4-ynyl)-*N*-methyl-1-naphthalene methanamine hydrochloride [1]. Terbinafine is an allylamine derivative reported to have a broad spectrum of antifungal activity. It is considered to act through inhibition of fungal sterol synthesis. It is fungicidal against dermatophytes, moulds, and certain dimorphic fungi and some yeast [2, 3]. Terbinafine is given by mouth as the hydrochloride in the treatment of dermatophyte infections of the skin and nails. It is also applied, as the hydrochloride, to the skin in dermatophytoses, in pityriasis versicolor and in cutaneous candidiasis. TRH is official in the United State Pharmacopoeia (USP) [4], in the British Pharmacopoeia (BP) [5], and in the European Pharmacopoeia (EP) [6].

Reviewing the literature revealed that, numerous analytical methods were developed for the assay of TRH, chromatographic methods including TLC [7–9], GC [10, 11], and HPLC. Different HPLC methods were reported for the assay of TRH either in dosage forms as cream [12–14], Tablets [15–17] and liniment [18], or in biological fluids [19–22]. Various spectrophotometric methods have also been used for the analysis of TRH [23–25]. This in addition to capillary electrophoresis [26, 27] and electrochemical methods [28–31].

Linezolid (Fig.1b); N-{[(S)-3-(3-Fluoro-4-morpholinophenyl)-2-oxo-5-oxazolidinyl] methyl}acetamide [1]. Linezolid is an oxazolidinone antibacterial effective against a range of aerobic gram-positive pathogenic bactéria, including methicillin-resistant Staphylococcus aureus (MRSA), glycopeptide-intermediate Staphylococcus aureus (GISA) and vancomycin-resistant enterococci (VRE) [2]. It is less active against Gram-negative bacteria, but has some in-vitro activity against Haemophilus influenzae, Legionella spp., Moraxella catarrhalis (Branhamella catarrhalis), Neisseria gonorrhoeae, and Pasteurella spp. It is not active against Acinetobacter spp., Enterobacteriacea or Pseudomonas spp. Oxazolidinone are bacteriostatic and act by inhibition of ribosomal protein synthesis, while RNA and DNA synthesís is not affected. Regarding LNZ, it is not yet official in any of the pharmacopoeia, but several methods have been reported for its determination, such as spectrophotometry [32–34], HPLC [35–37], TLC [38], capillary electrophoresis [39] and electrochemical analysis [40].

Spectrofluorimetry has been widely used in the determination of pharmaceutical compounds because it is a highly

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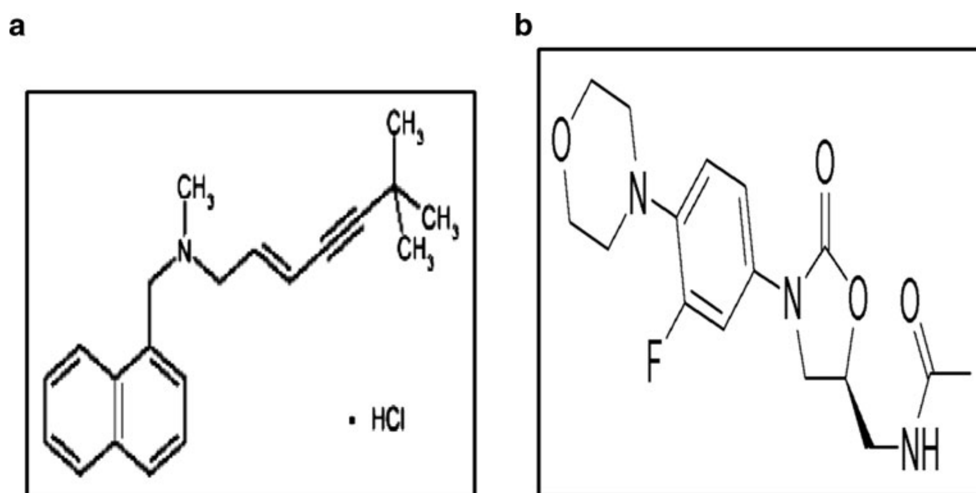

Fig. 1 Structural formulae of: **a** Terbinafine hydrochloride. **b** Linezolid

sensitive, selective, easily operated and economical technique. To the best of our knowledge, up till now nothing has been published concerning the specrofluorimetric determination of TRH. The current study aimed to develop and validate a simple, rapid and sensitive spectrofluorimetric methodology for the determination of TRH and LNZ utilizing their native fluorescence in aqueous medium. The proposed method was fully validated according to ICH guidelines, and successfully applied for the determination of the studied drugs in their different dosage forms.

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.
- A Consort NV P-901 pH –Meter (Belgium) was used for pH measurements.
- Ultrasonic bath, model SS 101 H 230, USA.
- Gemmy Vortex Mixer (Gemmy industrial corporation, Taiwan).
- Cellulose acetate syringe filters with 0.45 μm pore size (Gemma, Barcelona, Spain) was used for filtration of plasma samples.

Materials and reagents

- All chemicals were of analytical grade, and distilled water was used throughout the work.
- Terbinafine HCl was kindly provided by LKT Laboratories, lot# 2594805. The purity percentage of TRH was 100.17±1.19. The purity was established by applying the USP method (4).
- Linezolid was kindly provided by Glenmark Pharmaceuticals, Egypt, lot# 081001053.
- Sodium dodecyl sulphate (SDS; 95 %) and cetyl trimethyl ammonium bromide (CTAB; 99 %) were purchased from Winlab (UK).
- Methanol, acetonitrile and n-propanol were obtained from Sigma- Aldrich (Germany).
- Hydroxy propylβ-cyclodextrin (HP-β-CD) was obtained from Merck (Germany).
- Tween- 80, methyl cellulose, acetone, ethanol, glacial acetic acid, sodium acetate trihydrate, boric acid were all obtained from El-Nasr Pharmaceutical Chemical Co. (ADWIC; Egypt).
- Acetate buffer (0.2 mol/L, pH 3.0–5.5) and borate buffer (0.2 mol/L, pH (6.0–10.0) solutions were freshly prepared. SDS, CTAB, methylcellulose, HP-β-CD and Tween-80 were prepared as 0.1 % w/v aqueous solutions.
- Pharmaceutical preparations containing the studied drugs were purchased from different commercial sources in the local pharmacy.

  - Lamisil® 250 mg tablet (Produced by Novartis Pharma S.A.E Cairo-C.C.R. under license from Novartis Pharma AG., Basle, Switzerland), labeled to contain 250 mg of terbinafine as hydrochloride, batch # Y0018.
  - Lamisil® 125 mg tablet (Produced by Novartis Pharma S.A.E Cairo-C.C.R.111108 under license from Novartis Pharma AG., Basle, Switzerland), labeled to contain 125 mg of terbinafine as hydrochloride, batch # Y0002.
  - Lamisil® 1 % cream (Produced by Novartis Pharma S.A.E Cairo-C.C.R. 111108 under license from Novartis consumer Health SA, Nyon, Switzerland) batch # Y0150.
  - Lamisil® spray (Produced by Novartis Pharma SAS, Huningue, France, for Novartis Pharma AG. Basle,

Switzerland) labeled to contain 10 mg of terbinafine as hydrochloride/1gm spray, batch # H5264.

- Lamisil® derm gel (Produced by Novartis Pharma S.A.E Cairo-C.C.R. 111108 under license from Novartis consumer Health SA, Nyon, Switzerland) labeled to contain 10 mg of terbinafine/1gm gel, batch # Y0013.
- Averozolid 600 mg tablets (Produced by El-Obour Modern Phrmaceutical Co. for Averroes Pharma.), labeled to contain 600 mg of LNZ, batch # 107020.
- Zyvox ™ (Linezolid injection 600 mg/300 mL) (Produced by Fresenius Kabi Norge, Halden, Norway.), labeled to contain 2 mg of LNZ/mL, batch # 11L27Z35.

Standard Solution

Stock solution of 400.0 μg/mL of TRH and LNZ were prepared by dissolving 10 mg of each in 25 mL methanol with the aid of an ultrasonic bath. Working standard solutions of 1.0 μg/mL for TRH and 10.0 μg/mL for LNZ were prepared by appropriate dilution of the stock solutions with distilled water. Solutions of TRH were protected from light with aluminium foil. All solutions were stored in the refrigerator and found to be stable for at least 10 days without alteration.

Construction of Calibration Graph

Accurately measured aliquots of the suitable drug working standard solutions were transferred into a series of 10 mL volumetric flasks so that the final concentration was in the range of 20.0 150.0 ng/mL for TRH and 0.5–5.0 μg/mL for LNZ then completed to the volume with distilled water . The fluorescence intensity was measured at 336 nm after excitation at 275 nm for TRH and at 375 nm after excitation at 254 nm for LNZ . The relative fluorescence intensity was plotted against the final concentration of the drug. Alternatively, the corresponding regression equations were derived.

Procedures for Tablets

An accurately weighed quantity of the mixed contents of 10 powdered tablets equivalent to 10.0 mg of either TRH or LNZ was transferred into a 25 mL volumetric flask and about 15 mL of methanol were added. The contents of the flask were sonicated for 30 min, completed to the mark with the same solvent and filtered through cellulose acetate syringe filter. Further dilution with distilled water was performed to get working standard solution to be assayed by subjecting to the general procedure as described under "*construction of calibration graph*". The nominal content was calculated either from a previously plotted calibration graph or using the corresponding regression equation.

Analysis of TRH in Cream and Gel

An accurately weighed quantity of the cream or gel equivalent to 10.0 mg TRH was transferred into a clean dry 25 mL beaker and about 15 mL of methanol were added. The contents of the beaker were sonicated for 30 min, and then quantitatively transferred into 25 mL volumetric flask, completed to the mark with the same solvent, cooled in ice bath to solidify the base and filtered through cellulose acetate syringe filter. Further dilution with distilled water was performed to get working standard solution to be assayed by subjecting to the general procedure as described under "*construction of calibration graph*". The nominal content was calculated either from a previously plotted calibration graphs or using the corresponding regression equations.

Analysis of TRH in Spray

An accurately weighed quantity of the spray equivalent to 10.0 mg TRH was transferred into a clean dry 25 mL beaker and about 15 mL of methanol were added. The contents of the beaker were sonicated for 30 min, and then quantitatively transferred into 25 mL volumetric flask, completed to the mark with the same solvent. Further dilution with distilled water was performed to get working standard solution to be assayed by subjecting to the general procedure as described under "*construction of calibration graph*". The nominal content was calculated either from a previously plotted calibration graphs or using the corresponding regression equations.

Analysis of LNZ in I.V Solution

An accurately measured volume of the I.V solution equivalent to 10.0 mg LNZ was transferred into 25 mL volumetric flask and about 15 mL of methanol were added. The contents of the flask were sonicated for 30 min, completed to the mark with the same solvent. Further dilution with distilled water was performed to get working standard solution to be assayed by subjecting to the general procedure as described under "*construction of calibration graph*". The nominal content was calculated either from a previously plotted calibration graph or using the corresponding regression equation.

Analysis of TRH and LNZ in Spiked Human Plasma Samples

One mL aliquots of human plasma were transferred into a series of small screw capped centrifugation tubes and spiked with different concentrations of TRH (0.5–1.0 μg/mL) or

LNZ (15–20 μg/mL), then 1 mL of 1.0 M NaOH solution was added in case of TRH to liberate the free terbinafine base. The samples were extracted by vortex mixing with 3 x 3 mL of ethyl acetate for 2 min then centrifugation for 30 min at 3,000 rpm. The combined ethyl acetate extract were collected in small beakers and evaporated over night at room temperature in a gas hood. The residues were reconstituted with water and quantitatively transferred into a series of 25 mL volumetric flasks. The volumes were completed with water; the solutions were mixed well and filtered through cellulose acetate syringe filter. A blank experiment was carried out simultaneously. The relative fluorescence intensity was plotted against the final concentration of the drug. Alternatively, the corresponding regression equations were derived.

Results and Discussion

Both TRH and LNZ were found to exhibit intense native fluorescence in aqueous solution at 336 nm after excitation at 275 nm (Fig. 2a), and at 375 nm after excitation at 254 nm (Fig. 2b), respectively. As a consequence, we aimed to utilize these emission bands, in order to explore a new methodology for the analysis of TRH and LNZ in different pharmaceutical preparations.

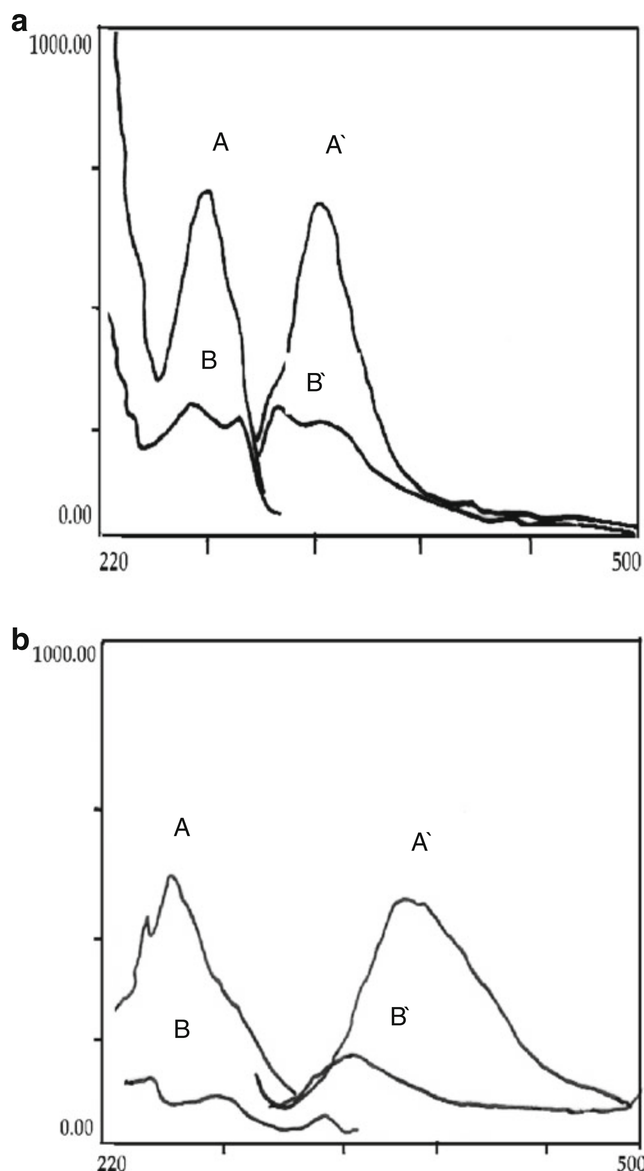

Fig. 2 Fluorescence spectra of: **a** Excitation and (A′) Emission spectra of (0.1 μg/mL) of TRH in water. **b** Excitation and (B′) Emission spectra of water. **a** Excitation and (A′) Emission spectra of (1.0 μg/mL) of LNZ in water. **b** Excitation and (B′) Emission spectra of water

Optimization of Experimental Conditions

- Effect of different organized media.

  The fluorescence properties of TRH and LNZ in various organized media were studied, using anionic surfactant (SDS), cationic surfactant (CTAB), non-ionic surfactant (Tween-80) and different macromolecules, CTAB, methyl cellulose and HP-β-CD where 1 mL of each surfactant(0.1 % w/v) was added to the aqueous solution of the drug(final concentration 20 ng/mL for TRH and 1.0 μg/mLfor LNZ). For TRH, Tween-80 caused a very slight increase of its RFI, while, SDS, CTAB, methyl cellulose, and HP-β-CD caused decrease in the RFI of the drug (Fig. 3a). In the case of LNZ, Tween-80 caused a very slight increase of tits RFI, where CTAB caused a very slight decrease in the RFI of LNZ and all the other organized media studied caused significant decreases in the RFI of the drug (Fig. 3b).

  It is obvious from the results (Fig. 3) that the presence of surfactants resulted in no significant effect or may decreased fluorescence intensity. Therefore, no surfactant was used in this work.

- Effect of pH.

  The influence of pH on the fluorescence of TRH and LNZ was studied using different types of buffers covering the whole pH range, such as 0.2 mol/L acetate buffer over the pH range 3.0–5.5 and 0.2 mol/L borate buffer over the pH range 6.0–10.0. For both drugs the use of buffer did not enhance the RFI over the entire pH range studied. It was found that maximum RFI was achieved in aqueous solution without the addition of any buffer.

- Effect of diluting solvent

  The effect of different diluting solvents on the RFI of TRH and LNZ was investigated using water, ethanol, methanol, acetonitrile, n-propanol and acetone. It was found that water was the best solvent for dilution, as it gave the highest RFI and the lowest blank reading

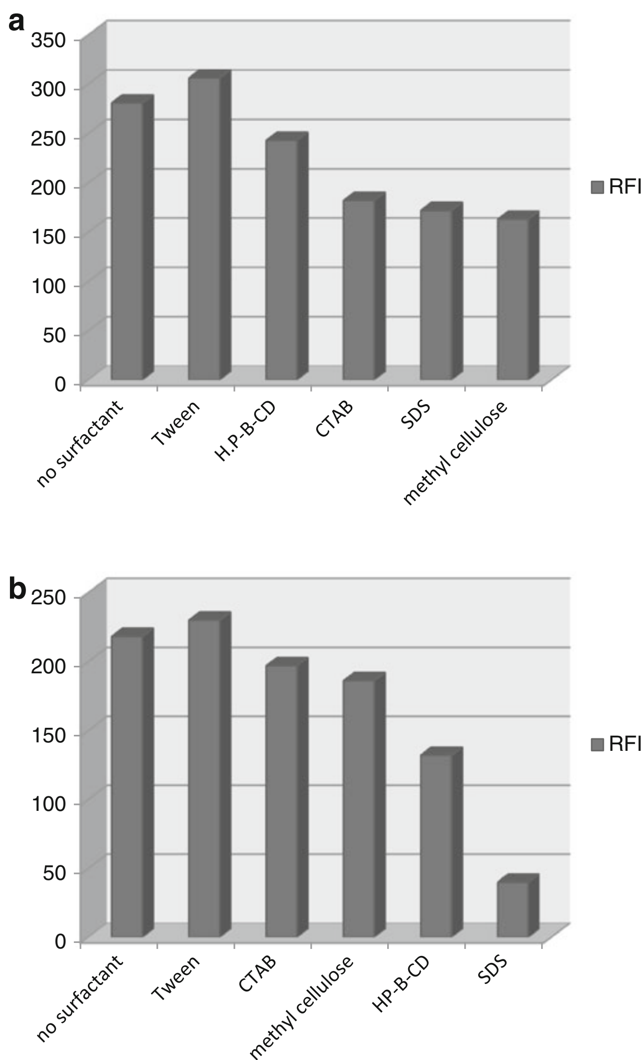

Fig. 3 **a** Effect of the type of organized media (1 mL 0.1 % solution of each) on RFI of TRH (0.05 μg/mL). **b** Effect of the type of organized media (1 mL 0.1 % solution of each) on RFI of LNZ (1.0 μg/mL)

(Table 1). Ethanol caused a very slight increase in the RFI of LNZ and did not affect the RFI of TRH.

Distinct and sharp decrease in the relative fluorescence intensities of both drugs was observed when using methanol, acetonitrile, acetone or n-propanol.

- Effect of time

The effect of time on the RFI of the two drugs was also studied. It was found that the fluorescence intensity remained stable for more than 2 h.

- Effect of temperature

Another factor that affects the fluorescence intensity is temperature. The effect of temperature was studied in the range 40–100 °C in a thermostatically controlled water bath. It was found that increasing the temperature resulted in a decrease in the RFI. This effect can be explained by higher internal conversion as the temperature increases, facilitating nonradiative deactivation of the excited singlet state [41]. Therefore, all the experiments were carried out at room temperature.

Table 1 Effect of different diluting solvents on the RFI of the studied drugs

Diluting solvent	RFI	
	TRH (50 ng/mL)	LNZ(1.0 μg/mL)
H_2O	283	218
Ethanol	233	240
Methanol	85	20
Acetonitrile	30	0
Propanol	25	Very high blank reading
Acetone	0	0

Validation of the Method

The validity of the method was checked by testing linearity, specificity, accuracy, repeatability and precision according to ICH recommendations [42].

Linearity

Assessment of linearity of the assay method was performed by analysing sex sets for each drug (standard calibration plots). The fluorescence vs. concentration plots were linear over the range 20–150 ng/mL for TRH and 0.5–5.0 μg/mL for LNZ. Linear regression

Table 2 Analytical performance data for the determination of the studied drugs by the proposed method

Parameter	TRH	LNZ
Linearity range	20.0–150.0 (ng/mL)	0.5–5.0 (μg/mL)
1Intercept (*a*)	73.59	84.64
Slope (*b*)	4.13	132.53
Correlation coefficient (*r*)	0.9998	0.9998
S.D. of residuals ($S_{y/x}$)	4.22	5.43
S.D. of intercept (S_a)	3.87	4.22
S.D. of slope (S_b)	0.04	1.39
S.D.	1.16	1.27
% RSD [a]	1.16	1.27
% Error [b]	0.47	0.52
LOD[c]	3.09 (ng/mL)	0.11 (μg/mL)
LOQ[d]	9.39 (ng/mL)	0.32 (μg/mL)

[a] Percentage relative standard deviation

[b] Percentage relative error

[c] Limit of detection

[d] Limit of quantitation

Table 3 Assay results for the determination of the studied drugs in pure form by the proposed and comparison methods

Compound	Proposed method			Comparison method [4, 37]		
TRH	Amount taken (ng/mL)	Amount found (ng/mL)	% Found	Amount taken (μg/mL)	Amount found (μg/mL)	% Found
	20.0	20.208	101.04	50.0	50.678	101.36
	50.0	50.732	101.46	100.0	98.984	98.98
	80.0	79.077	98.85	200.0	200.339	100.17
	100.0	98.699	98.70			
	120.0	120.988	100.82			
	150.0	150.301	100.20			
Mean			100.18			100.17
± S.D.			1.16			1.19
t-test			0.01			(2.37)
F-test			1.05			(5.79)
LNZ	Amount taken (μg/mL)	Amount found (μg/mL)	% Found	Amount taken (μg/mL)	Amount found (μg/mL)	% Found
	0.5	0.493	98.64	16.0	15.869	99.18
	1.0	1.006	100.63	24.0	24.262	101.09
	2.0	2.033	101.63	32.0	31.869	99.59
	3.0	2.983	99.44			
	4.0	3.942	99.54			
	5.0	5.043	100.86			
Mean			99.96			99.95
± S.D.			1.27			1.01
t-test			0.004			(2.37)
F-test			1.59			(19.29)

N.B. Each result is the average of three separate determinations

The figures between parentheses are the tabulated t and F values at $P=0.05$ [43]

Table 4 Accuracy and precision data for the determination of the studied drugs by the proposed method

Parameter		TRH (ng/mL)			LNZ (μg/mL)		
		50.0	80.0	120.0	1.0	2.0	3.0
Intraday	% Found	101.46	98.85	100.82	100.63	101.63	99.44
		100.47	100.23	97.69	98.39	100.22	100.49
		101.90	99.54	99.35	100.97	97.08	98.05
	Mean	101.28	99.54	99.29	100.0	99.64	99.33
	S.D.	0.73	0.69	1.57	1.40	2.33	1.22
	% RSD	0.72	0.69	1.58	1.40	2.34	1.23
	% Error	0.42	0.40	0.91	0.80	1.35	0.71
Interday	% Found	101.46	98.85	100.82	100.63	101.63	99.44
		99.17	100.13	99.13	102.62	101.71	99.17
		98.79	97.93	98.69	101.74	99.57	100.69
	Mean	99.81	98.97	99.98	101.66	100.97	99.77
	S.D.	1.44	1.11	1.20	1.0	1.21	0.81
	% RSD	1.45	1.12	1.20	0.98	1.20	0.81
	% Error	0.84	0.64	0.69	0.57	0.69	0.47

N. B. Each result is the average of three separate determinations

Table 5 Assay results for the determination of the studied drugs in their different dosage forms by the proposed method

Parameter	Proposed method			Comparison method [4, 37]		
	Amount taken (ng/mL)	Amount found (ng/mL)	% Found	Amount taken (μg/mL)	Amount found (μg/mL)	% Found
Lamisil ®250 mg tablet	50.0	49.585	99.17	25.0	24.697	98.79
	80.0	81.0	101.25	50.0	50.317	100.63
(TRH 250 mg/tablet)	120.0	121.047	100.87	200.0	204.946	102.47
Mean			100.43			100.63
± S.D.			1.11			1.84
t-test			0.16			(2.78)
F-test			2.76			(19.0)
Lamisil ®125 mg tablet (TRH 125 mg/tablet)	50.0	50.338	100.68	25.0	25.713	102.85
	80.0	82.047	102.56	50.0	50.312	100.62
	120.0	123.205	102.67	200.0	202.759	101.38
Mean			101.97			101.62
± S.D.			1.12			1.13
t-test			0.39			(2.78)
F-test			1.03			(19.0)
Lamisil ®spray (TRH 10 mg/1gm spray)	50.0	49.117	98.23	25.0	24.838	99.35
	80.0	81.20	101.50	50.0	50.130	100.26
	120.0	120.624	100.52	200.0	201.68	100.84
Mean			100.08			100.15
± S.D.			1.68			0.75
t-test			0.06			(2.78)
F-test			4.99			(19.0)
Lamisil ®cream (TRH 10 mg/1gm cream)	50.0	49.664	99.33	25.0	24.915	99.66
	80.0	79.813	99.77	50.0	49.910	99.82
	120.0	119.199	99.33	200.0	201.780	100.89
Mean			99.47			100.12
± S.D.			0.25			0.67
t-test			1.57			(2.78)
F-test			6.93			(19.0)
Lamisil ®gel (TRH 10 mg/1gm gel)	50.0	49.855	99.71	25.0	25.388	101.55
	80.0	80.374	100.47	50.0	50.030	100.06
	120.0	121.047	100.87	200.0	198.260	99.13
Mean			100.35			100.25
± S.D.			0.59			1.22
t-test			0.13			(2.78)
F-test			4.29			(19.0)
Averozolid 600 mg tablet (600 mg LNZ/tablet)	1.0 μg/mL	0.981	98.12	16.0	15.887	99.29
	2.0 μg/mL	1.979	98.96	24.0	24.226	100.94
	3.0 μg/mL	2.937	97.89	32.0	31.887	99.65
Mean			98.32			99.96
± S.D.			0.56			0.87
t-test			2.74			(2.78)
F-test			2.37			(19.0)
Zyvox infusion (2 mg LNZ/mL)	1.0 μg/mL	0.977	97.67	16.0	16.223	101.40
	2.0 μg/mL	1.987	99.36	24.0	23.553	98.14
	3.0 μg/mL	2.937	97.91	32.0	32.223	100.70
Mean			98.31			100.08
± S.D.			0.91			1.72
t-test			1.57			(2.78)
F-test			3.52			(19.0)

N.B. Each result is the average of three separate determinations

The figures between parentheses are the tabulated t and F values at $P=0.05$ [43]

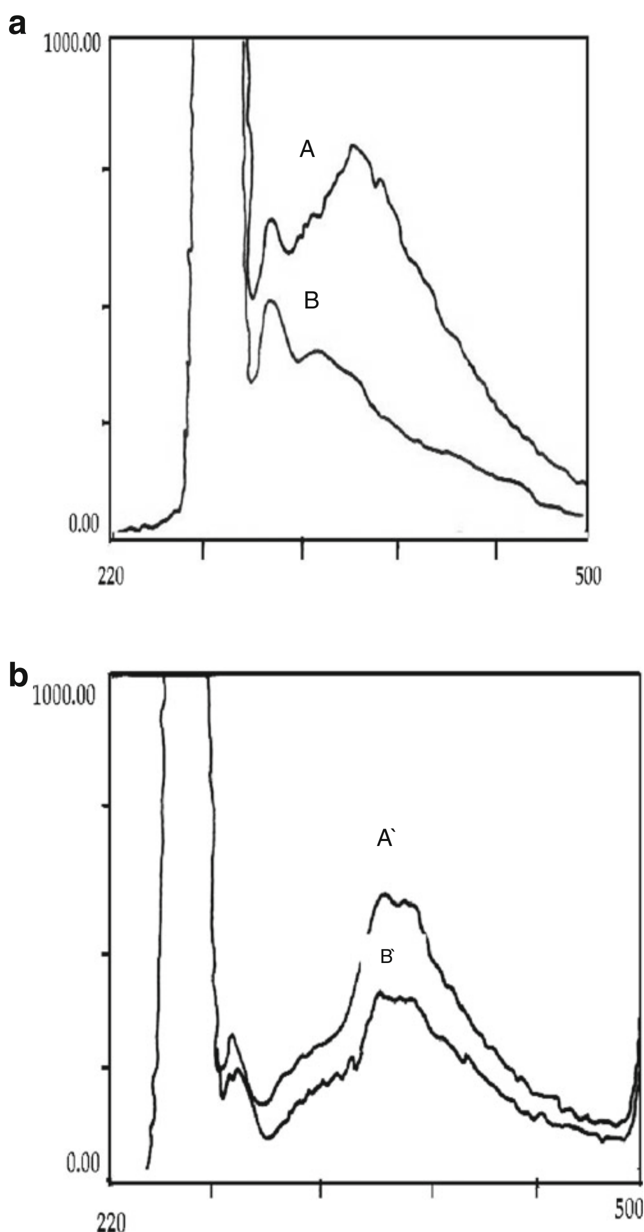

Fig. 4 Fluorescence spectra of the studied drugs in plasma: **a** Emission spectrum of (0.04 μg/mL) of TRH in plasma. **b** Emission spectrum of blank plasma after excitation at 275 nm. (A′) Emission spectrum of (0.72 μg/mL) of LNZ in plasma. (B′) Emission spectrum of of blank plasma after excitation at 254 nm

analysis of the data gave the following equations:

$$RFI = 73.59 + 4.128\ C\ (r = 0.9998)\ \text{for TRH}$$

$$RFI = 84.64 + 132.53\ C\ (r = 0.9998)\ \text{for LNZ}$$

Where: RFI is the relative fluorescence intensity, C is the concentration of the drug in ng/mL for TRH and in μg/mL for LNZ and r is the correlation coefficient.

Statistical analysis [43] of the data gave high value of the correlation coefficient (*r*) of the regression equation, small values of the standard deviation of residuals ($S_{y/x}$), of intercept (S_a), and of slope (S_b), and small value of the percentage relative standard deviation and the percentage relative error (Table. 2). These data proved the linearity of the calibration graph.

Limits of Quantification (LOQ) and Limits of Detection (LOD)

The limits of quantitation (LOQ) were determined by establishing the lowest concentrations that can be measured according to the ICH Q2 (R1) recommendation [42] below which the calibration graph is non-linear. The limits of detection (LOD) were determined by evaluating the lowest concentrations of the analytes that can be readily detected. The results are also summarized in Table 2.

The values of LOQ and LOD were calculated according to the following equation [42]:

$$LOQ = 10Sa/b$$

$$LOD = 3.3Sa/b$$

Where Sa is the standard deviation of the intercept of the regression line and b is the slope of the calibration graph.

Accuracy and Precision

Statistical analysis [43] of the results obtained by the proposed and reference methods [4, 37] using Student's *t*-test and variance ratio F-test showed no significant differences between the two methods regarding accuracy and precision (Table 3).

The intraday precision was evaluated by determination of three concentrations of each drug in pure forms on three successive occasions. The interday precision was also evaluated through replicate analysis of three concentrations for a period of 3 successive days. The results of intraday and interday precision are summarized in Table 4. The relative standard deviations were found to be very small indicating reasonable repeatability and intermediate precision of the proposed methods.

Selectivity

The selectivity of the method was investigated by observing any interference encountered from common excipients in different formulations. It was shown that these compounds did not interfere with the results of the proposed method.

Table 6 Assay results for the determination of TRH and LNZ in spiked human plasma using the proposed method

Parameter	TRH			LNZ		
	Amount taken (ng/mL)	Amount found (ng/mL)	% Found	Amount taken (μg/mL)	Amount found (μg/mL)	% Found
	20.0	17.446	87.23	0.56	0.595	106.21
	24.0	25.220	105.08	0.64	0.621	97.03
	30.0	33.157	110.52	0.72	0.654	90.81
	40.0	38.178	95.44	0.80	0.851	106.31
Mean			99.57			100.09
S.D.			10.32			7.56
% RSD			10.37			7.76
% Error			5.16			3.78

Pharmaceutical Applications

The proposed method was applied to the determination of TRH in different commercially available dosage forms, such as tablets, cream, derm-gel and spray; also, it was applied to the determination of LNZ in its tablet and injection formulations (Table 5). The results shown in Table 5 are in good agreement with those obtained using the reference and comparison methods [4, 37]. Statistical analysis of the results obtained using Student's *t*-test and variance ratio F-test [43] revealed no significant difference between the performance of the two methods regarding the accuracy and precision, respectively.

Application to Spiked Human Plasma

TRH is well absorbed from the gastrointestinal tract. The bioavailability is about 40 % because of first-pass hepatic metabolism. Mean peak plasma concentrations of about 1 μg/mL occur within 2 h of a single oral dose of 250 mg. Steady state concentrations are about 25 % higher than those seen after a single dose and are reached in 10 to 14 days. Terbinafine is extensively bound to plasma proteins [2].

LNZ is well absorbed, with a bioavailability of approximately 100 % in healthy volunteers [2, 44]. This characteristic is a major benefit, allowing this agent to be used early intravenously, then switching to oral, or indeed even to commence treatment of infection with oral therapy. After oral doses of 600 mg, steady-state peak serum concentrations (C_{max}) are 15–27 μg/mL and are reached 0.5–2 h after administration [44–49]. The level of plasma protein binding is 31 % and the volume of distribution approximates to the total body water content of 40–50 L.

Figure 4 shows TRH and LNZ spectra obtained from spiked human plasma. Table (6) shows the results obtained from spiked plasma. Under the above described experimental conditions, a linear relationship was established by plotting the relative fluorescence intensity against the drug concentration, where linear regression analysis of the data gave the following equations:

$$RFI = 34.291 + 6.174\ C\ (r = 0.956)\ \text{for TRH}$$

$$RFI = -39.40 + 305.0\ C\ (r = 0.889)\ \text{for LNZ}$$

Where: RFI is the relative fluorescence intensity, C is the concentration of the drug in ng/mL for TRH and in μg/mL for LNZ and r is the correlation coefficient. (Table 6)

Conclusion

A simple and sensitive spectrofluorimetric method was developed for the determination of TRH and LNZ. The proposed method is rapid, less time-consuming and does not require the elaborate treatment associated with chromatographic methods; moreover, it is sensitive, with no need for derivatization reactions. By virtue of its simplicity and rapidity, the proposed method could be applied to the analysis of the two drugs in their different dosage forms. The method was extended to the determination of the studied drugs in spiked human plasma. The proposed method is a non–pollutant methodology, since no organic solvents are used in the procedure

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