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Original Article

Fluorimetric Methods for the Determination of Terazosin HCl in Drug Substance and Dosage Forms

Taghreed A. Mohamed , *Nahla S.Ismail

National Organization for Drug Control and Research, P.O. Box 29, Giza, Cairo, Egypt

Abstract

Determination of terazosin hydrochloride dihydrate (TRZ) in drug substance and tablets was studied by fluorimetric techniques. The fluorimetric methods are based on: (1) measurement of the native fluorescence of the drug in water at 750 nm after excitation at 330 nm. (2) sensitizing the native fluorescence by formation a binary complex of drug with aqueous uranyl acetate (0.1% w/v) at the same E_x / E_m. under the described condition. the proposed methods were applicable over the concentration range of (10 -1000 and 0.5-12 ng mL^{-1}) with good correlation ((r^2=0.9982 and 0.9987), limit of detection of (3.47 and 0.198 ng/mL) and a lower limit of quantification of (10.5 and 0.6 ng mL^{-1}) for method (1) and (2), respectively. The described methods were successfully applied for the determination of TRZ in its commercial tablets without interference from common excipients.

Keywords: fluorimetric determination, sensitizing the native fluorescence, Terazosin HCl, uranyl acetate.

Corresponding Author: Nahla S.Ismail, National Organization for Drug Control and Research, P.O. Box 29, Giza, Cairo, Egypt
Tel: (02) 01003432900
E-Mail: sindhusa@gmail.com
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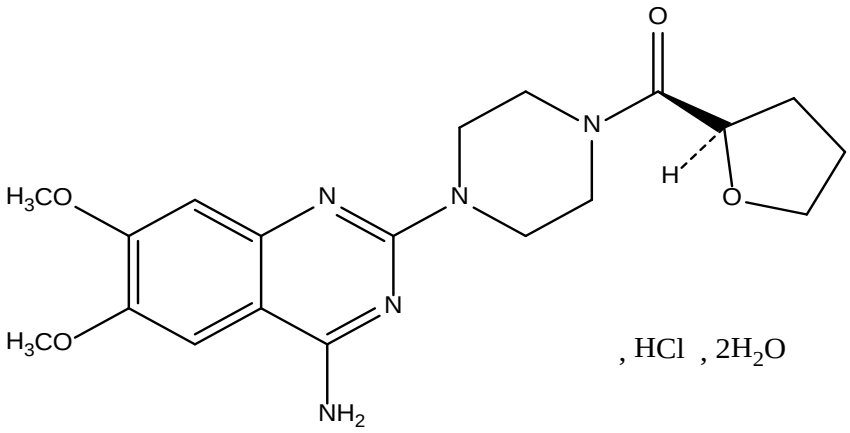
1. Introduction

Terazosin HCl has the IUPAC name of 1-(4-Amino-6,7-dimethoxy-2-quinazolinyl)-4-[(tetrahydro-2furanyl) carbonyl] piperazine hydrochloride dihydrate. Scheme (1). It's molecular formula is $C_{19}H_{25}N_5O_4$, HCl, H_2O. Terazosin HCl (TRZ.HCl) is an alpha$_1$-adrenoceptor blocker with actions similar to those of prazosin, but a longer duration of action. It is used in the management of hypertension and in benign



Scheme 1. Structural formula of terazosin HCl.

prostatic hyperplasia to relieve symptoms of urinary obstruction.

An acid-base titrimetric method is recommended for the determination of TRZ.HCl as drug substance in British pharmacopoeia [1], but its formulation is not officially up till now in any pharmacopoeia. Owing to the therapeutic importance of TRZ.HCl, various analytical procedures have been established for its quantitative determination in drug substance, drug products and human plasma.

These procedures include high performance liquid chromatography (HPLC) with fluorescence detection [2–4], with mass spectrometric or UV detection [5,6], X-ray fluorescence spectrometry based on the formation of ion-pair associates with zinc thiocyanate [7], spectrophotometric [8-13] chemometric and capillary zone electrophoresis methods [14,15],.potentiometric [16] voltammetric technique[17] and fluorimetry [18].

Native fluorescence and sensitizing the native fluorescence by formation of a binary complex of the drug with uranyl acetate were not reported in these articles.

Uranyl acetate is a yellow free flowing, crystalline, water soluble uranium compound. It has been used as a chromogenic agent for the spectrophotometric determination of different drugs through complex formation [19, 20]. It is also used as a fluorescent indicator for TLC chromatographic determination of complexing agents [21]. It has quenching effect on the fluorescence intensity by the formation of binary complexes [22]. The sensitization effect of uranyl acetate on the native fluorescence

through the formation of binary complex was studied.

2. Materials and Methods

2.1. Reagents and Materials

All chemicals and reagents used were of analytical reagent grade. Bidistilled water was used throughout all experiments. The raw material terazosin hydrochloride (TRZ.HCl) was kindly provided by Abbott/Kahira, Egypt Company for Pharmaceutical Industry. Its purity was found to be 99.80%±0.19% according to the official titrimetric method [1]. Itrin tablets B.N. 19503 labeled to contain 5 mg of terazosin hydrochloride, manufactured by Abbott Pharmaceutical Industries, and Prostasin tablets B.N. P4691212, labeled to contain 5 mg of terazosin hydrochloride, manufactured by (Pharco). Uranium acetate reagent (BDH chemicals) 0.1% w/v was freshly prepared in water.

2.2. Drug Substance

Stock standard solutions for two flourimetric methods were prepared by dissolving TRZ.HCl 0.1 mg mL^{-1} in water. The working standard solutions within linearity range were prepared using water.

2.3. Drug Products (Tablets)

(a) Ten tablets were accurately weighed and finely powdered. The required amount from the tablets powder of each pharmaceutical product (Itrin®, and Prostasine, 5 mg/tablet) were weighed, and then dissolved in 50 mL of bidistilled water by sonication for 10 min. The solution mixture was shacked in a mechanical shaker and the mixture was filtered then transferred accurately to 50 mL measuring flask, completed to the mark with bidistilled water shacked and finally determined by the proposed sensors.

An Aliquot of analyte solution (a) containing 10 mg of drug was pipette into a 100-mL beaker, and the solution was diluted to 100 mL with bidistilled water. The procedure was completed as mentioned under general procedure. The nominal content of the tablets was determined either from the calibration curve or using the corresponding regression equation.

2.4. Apparatus

Shimadzu RF -1501 spectofluorimeter, equipped with Xenon arc lamp, using quartz cell (1 x1 x 4.5 cm).

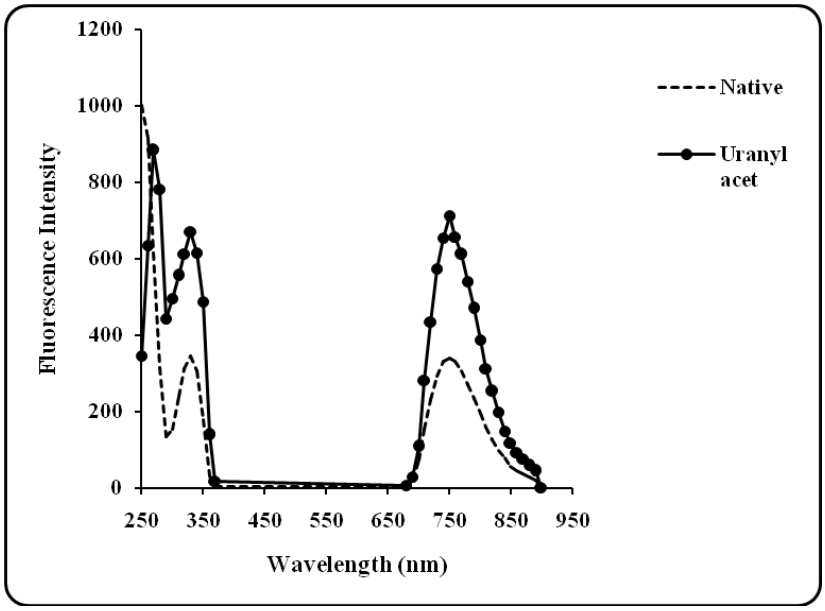


Figure 1. Excitation and Emission spectra of TRZ. solution (0.4 µg mL^{-1}), Excitation and Emission spectra after reaction with uranyl acetate (0.1% w/v) and TRZ.(4 ng mL^{-1}).

2.5. Fluorimetric Determination of TRZ.HCl

2.5.1. Native Fluorescence Method

The native fluorescence study of TRZ.HCl: Aliquots containing different amounts of the analyses, between 0.1-10 µg mL^{-1}(100-10000 ng mL^{-1}) were pipette into 10 mL calibrated flasks, and diluted to the mark with water. The fluorescence emission was measured at 750 nm using excitation wavelength of 330 nm, against a blank solution.

2.5.2. Uranyl Acetate Method

For sensitizing method : Aliquots corresponded to 5-120 ng TRZ.HCl were transferred from stock solution to a series of 10-mL volumetric flasks, followed by adding 2 mL 0.1% w/v of uranyl acetate and completed to the mark with water. The fluorescence intensity of solution was measured at 750 nm with excitation at 330 nm against a blank prepared similarly.

3. Results and Discussion

3.1. Flourimertric Method

3.1.1. Fluorescence Spectral Properties of TRZ. HCl

TRZ.HCl is freely soluble in water; the solution exhibited an intense native fluorescence in different media, such as water, 0.1 M HCl, 0.1 M NaOH, and methanol. The highest fluorescence intensity

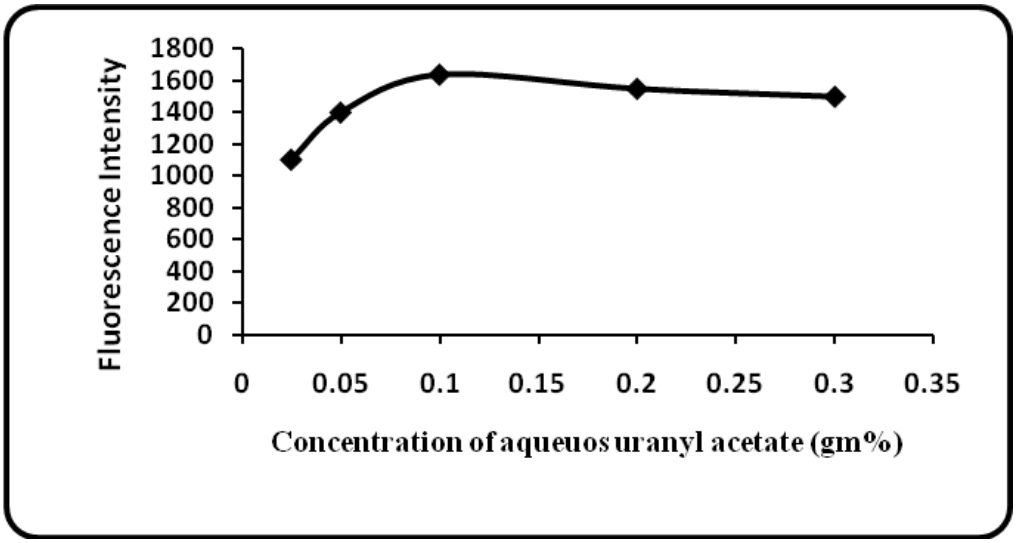


Figure 2. Effect of concentration of aqueous uranyl acetate on the sensitization fluorescence after reaction with TRZ. (10 ng ml^{-1}).

was obtained in water; at λ emission 750 nm upon using 330 nm as λ excitation.

Uranyl acetate solution has high fluorescence intensity [23] and when added to the studied drug its fluorescence intensity increases. This behavior has been observed due to formation of binary complex between TRZ. HCl and uranyl acetate, which confers structural rigidity and enhancing fluorescence, Figure (1).

Optimum conditions for binary complex formation were studied. Different concentrations of aqueous uranyl acetate ranging from (0.025-0.3 % w/v) were tried and the most suitable volume was also used Figure (2). It was found that 2 ml of 0.1% w/v aqueous solution was adequate for best results. The fluorescence signal was linearly related to the concentration in the range (10-1000 ng ml^{-1}) for native and (0.5-12 ng ml^{-1}) for complex with mean percentage recoveries 100.15 ± 0.226 and 100.41 ± 0.234, respectively. The binary complex is formed immediately and remains stable at least 6 h. as shown in (Table 1).

3.1.2. Determination of Fluorescence Quantum Yield of TRZ.HCl and Uranyl Acetate

Diluted solution of quinine sulfate dissolved in 0.05 mol L^{-1} sulfuric acid with fluorescence quantum yield of 0.55 was used as reference reagent, and an equation

Table 1. Quantitative parameters and statistical data of the regression equations for the fluorimetric analysis of TerazocineHCl in their drug substances using native fluorescing and sanitization by using uranyl acetate.

Parameters	**Terazosin HCl**	
	Native method	**Uranyl acetate method**
$\lambda_{Ex/Em}$	330 / 750	330 / 750
Linearity range (ng ml $^{-1}$)	10 -1000	0.5 – 12
Regression equation [a]	F = 0.843 C – 0.196	F = 166.3C – 30.72
Slop	0.8339	166.313
SD of slope	0.0000412	0.00182
S E of slope	0.014491	2.44933
Confidence limit of slope [b]	0.7967 – 0.87122	160.017 – 172.609
Intercept	- 0.1960	- 30.7232
SD of intercept	0.0000433	0.003967
S E of intercept	7.43457	16.7726
Confidence limit of [b]	- 19.307 – 18.915	-73.8387 - 12.3923
Correlation coefficient (r)	0.9985	0.9987
SE of (r)	13.2159	26.4369
Accuracy (Mean [c] ± RSD %)	100.15 ± 0.226	100.41 ± 0.234
Precision		
Repeatability	100.2 0 ± 0.216	100.07 ± 0.152
Intermediate precision	100.10 ± 0.271	100.06 ± 0.096
LOD (ng ml^{-1})	3.47	0.198
LOQ (ng ml^{-1})	10.5	0.6

shown was used to calculate the fluorescence quantum yield of TRZ. [24].

Yu = Ys (Fu / Fs) (As / Au). (1)

Yu and Ys referred to the fluorescence quantum yield of TRZ. and quinine sulfate, respectively; Fu and Fs represented the integral fluorescence intensity of TRZ. and quinine sulfate, respectively; Au and As referred to the absorbance of TRZ. and quinine sulfate at the excited wavelength, respectively. The concentration was selected so that the absorbance was less than 0.05 to minimize error arising from inner effect [25]. The fluorescence quantum yield found to be 0.42 of the TRZ.HCl with uranyl acetate complex.

3.1.3. Method Validation

Validation of the proposed methods was assessed according to USP guidelines [26] for linearity and range, accuracy, precision, detection limit, quantitation limit and robustness.

Table 2. Results of standard addition method.

Method	Concentration of drug in formulation (µg mL^{-1})	Concentration of pure drug added (µg mL^{-1})	% level of pure drug added	Total concentration of drug found (µg mL^{-1})	% Analytical recovery ± SD
Native method	5	0.02	2	5.01	99.8±0.01
	5	0.2	20	5.21	100.2±0.15
	5	0.8	80	5.82	100.4±0.12
Uranyl acetate method	5	0.004	0.4	5.003	99.9±0.03
	5	0.008	0.8	5.009	100.2±0.08
	5	0.012	1.2	5.03	100.4±0.01

Each value is a result of three separated determination

3.1.3.1. Linearity and Range

The fluorescence concentration plot for the studied drug was linear over the range of 10 -1000 $ngml^{-1}$for native method and 0.5 – 12 ng mL^{-1} for uranyl acetate method, Linear regression analysis of the data gave the equations listed in Table (1).

The proposed methods were evaluated by studying the accuracy as percent relative error and precision as percent relative standard deviation, the results are abridged in Table 1.

3.1.3.2. Accuracy

As a part of determining accuracy of the proposed methods, different levels of drug concentrations were prepared from independent stock solution and analysed (N=6). Accuracy was assessed as the standard deviation and percentage relative standard deviation studies were found to be satisfactory Table 1. To give additional support to accuracy of the developed assay method, standard addition method was done. The percent recovery of the added pure drug was calculated as,

$$\% \text{ Recovery} = [(C_y - C_u) / C_a] \times 100$$

Where C_y is the total drug concentration measured after standard addition. C_u is the drug concentration in the formulation. C_a is the drug concentration added to the formulation Table (2).

3.1.3.3. Precision

Repeatability was determined by using different levels of drug concentration

(same concentration levels taken in accuracy study), prepared from independent stock solution and analysed (N=6) Table 1. Inter-day and intra- day variation and instrument variation were taken to determine intermediate precision of the proposed methods (N=6). The % relative standard deviation of the predicated concentrations from the regression equation was taken as precision Table (1).

of y-intercept of regression equation Table (1).

3.2. Analytical Application

The application of fluorimetric determination of TRZ.HCl in the drug substance and pharmaceutical products gives good results as shown in Tables (3, 4).

Table 3. Analysis TerazocineHCl in its drug substances using native fluorescing method and sanitization by using uranyl acetate method compared with theoffecial titrimetric method.

Parameters	**Fluorometric method**		
	Native method	**Uranyl acetate method**	**Reference method[1] BP**
Mean Recovery %	100.09	100.42	100.05
±RDS [a] %	0.268	0.169	0.129
Variance	0.072	0.029	0.016
SE	0.0346	0.069	0.053
t-test	0.63	4.25	(2.228)[b]
F-test	4.3	1.72	(5.1)[b]

3.1.3.4. Limit of Detection (LOD) and Limit Of Quantitation (LOQ)

The LOD and LOQ of the terazocin HCl by proposed methods were determined by using calibration standards. LOD and LOQ were calculated as 3.3σ/S and 10σ /S, respectively, where S is slope of the calibration curve and σ is standard deviation

3.3. Statistical Treatment of the Results

The calculated F values [27] were less than the tabulated F values where υ1 =6 and υ2 = 6 for batch condition at 95% confidence level. The t-test [27] was also done at 99.9% confidence level and the results are shown in Tables (3, 4). The applied method does not exhibit significant difference in comparison with the reference

Table 4. Analysis of terazosin HClin their drug products by the proposed (native fluorescing and formation of uranyl acetate complex) methods and compared with the reported method.

Parameters	**Itrein 5 mg**			**Prostasin 5 mg**		
	Fluorimetric method		Reported HPLC Method[6]	Fluorimetric method		Reported HPLC Method[6]
	Native method	Uranyl acetate method		Native method	Uranyl acetate method	
Mean*	100.2	100.08	100.1	100.5	100	99.36
± RDS %	0.224	0.237	0.225	1.11	0.48	0.61
Variance	0.050	0.056	0.051	1.23	0.23	0.372
SE	0.091	0.096	0.092	0.453	0.272	0.262
t- test	0.833	0.15	(2.228)[a]	2.1	1.91	(2.228)[a]
F-test	1.02	1.09	(5.1)[a]	3.3	1.61	(5.1)[a]

method [1, 6] which reflects the accuracy and precision of the proposed methods.

4. Conclusion

The two proposed spectrofluorimetric methods proved to be successful in routine quality control testing scince they provided a rapid, simple and low cost for the determination of TRZ.HCl in pure solutions and in pharmaceutical preparations without interference from common excipients.

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