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SPECTROFLUORIMETRIC DETERMINATION OF TOLTERODINE TARTARATE IN PURE FORM AND PHARMACEUTICAL PREPARATION

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ABSTRACT: A Simple, rapid, sensitive, accurate and precise spectrofluorimetric method was developed for the determination of tolterodine tartrate (TOL) in bulk powder and in pharmaceutical preparation. This method is based on measuring the native fluorescence of TOL in methanol at 313 nm after excitation at 285 nm using 1 ml of phosphate buffer pH 6. All variables that affect fluorescence intensity such as diluting solvents, pH, buffer and buffer volume were studied and optimized .The fluorescence-concentration plot was rectilinear over the range of 0.5-8 µg/ml with a lower detection limit (LOD) of 0.090 µg/ml and lower quantification limit (LOQ) of 0.273 µg/ml. The method was validated and successfully applied to the determination of TOL tablets with an average percent recovery ± RSD% of 100.57 ± 0.76. The obtained results were statistically compared with those of the reported method by applying t-test and F-test at 95% confidence level and no significant difference was observed regarding accuracy and precision.

INTRODUCTION: Tolterodine tartrate (**Fig. 1**) is 2-[(1R)-3-[Bis(1-methylethyl)amino]-1-phenyl propyl]-4-methylphenol [1]. It is a tertiary anti-muscarinic with actions similar to those of atropine. It is used in the management of urinary frequency, urgency and incontinence in detrusor instability [2]. Literature survey shows that several spectrophotometric [3-9] and chromatographic [10-18] methods for determination of tolterodine in pure form, pharmaceutical preparations and/or biological fluids have been reported.

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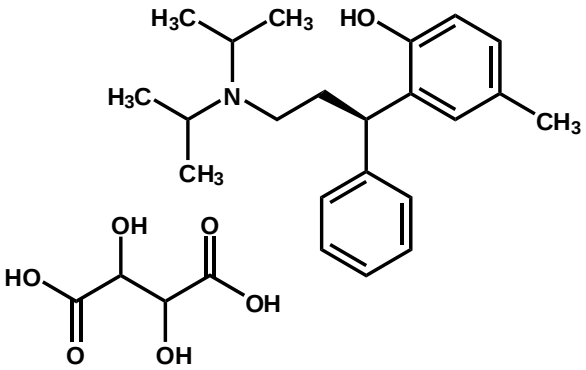

FIGURE 1: STRUCTURAL FORMULA OF TOLTERODINE TARTARATE

MATERIALS AND METHOD:

Apparatus:

- Jasco FP-6200 Spectrofluorometer (Japan), equipped with 150 Watt Xenon lamp, holographic gratting excitation and emission

monochromators for all measurements. Slit widths for both monochromators were set at 10 nm. A 1 cm quartz cell was used.

- Jenway, 3510 pH meter (Jenway, U.S.A.).

Materials and Reagents: All chemicals and reagents used throughout the work were of analytical grade.

- Tolterodine tartrate was kindly supplied by El-Kahira, Cairo, Egypt. The purity was assigned as 99.55 %.
- Detrusitol® tablets contain 2 mg tolterodine-L-tartrate. (B. No. - H885A, manufactured by Pharmacia & Upjohn) was obtained from commercial source.
- Water used throughout the procedures was freshly double distilled.
- Acetonitrile, methanol, ethanol, 1-propanol, chloroform, dichloroethane and tetrahydro-furan, all of HPLC grades [Sigma, Germany].
- Sodium hydroxide (El-Nasr Company, Egypt) prepared as 0.1 N aqueous solution.
- Hydrochloric acid (El-Nasr Company, Egypt) prepared as 0.1 N aqueous solution.
- Monobasic potassium phosphate, potassium chloride, boric acid, glacial acetic acid and sodium acetate trihydrate (El-Nasr Company, Egypt).
- Buffers of different pH values prepared as prescribed in US pharmacopeia [19]:

    1. Acetate buffer pH range from 4.1 to 5.5.
    2. Phosphate buffer pH range from 6 to 8.
    3. Alkaline borate buffer pH range from 8 to 10.

Standard Solution: A stock solution of TOL (200 µg/ml) was prepared by dissolving 20 mg of Tol in 50 ml of water and complete to 100 ml with water and was further diluted with the same solvent as appropriate. The standard solution was stable for 2 weeks when kept in the refrigerator [6].

Procedure:

Construction of the Calibration Curve (General Procedure): Different aliquots of TOL stock solution ranging from (5-80) µg were transferred to a 10 ml volumetric flasks and 1 ml of phosphate buffer pH 6 was added. The solutions were diluted with methanol to 10 ml and mixed well. The fluorescence intensity was measured at 313 nm ($\lambda_{excitation}$= 285 nm).The measured fluorescence intensity vs the final concentration in µg/ml were plotted to get the calibration graph. Alternatively, the regression equation was derived.

Analysis of Pharmaceutical Preparation: Weigh and pulverize 20 tablets. Transfer a weighed quantity of the powder equivalent to 20.0 mg of TOL into a small conical flask. Extract with 3 x 30 ml of water on three successive times each with 30 ml. Filter the extract into a 100 ml volumetric flask. Wash the conical flask with few mls of water. Pass the washings into the same conical flask and complete to the mark with the same solvent. Transfer aliquots covering the working concentration range into 10 ml volumetric flasks. Proceed as described under "General Procedure", adopting the method. Determine the nominal content of the tablets either from the calibration curve or using the corresponding regression equation.

RESULTS AND DISCUSSION:

Spectral Characteristics: TOL exhibits a native fluorescence in methanol and its emission can be measured at 313 nm ($\lambda_{emission}$) after excitation at 285 nm ($\lambda_{excitation}$). The emission and excitation spectra of TOL in methanol are shown in **figure 2.**

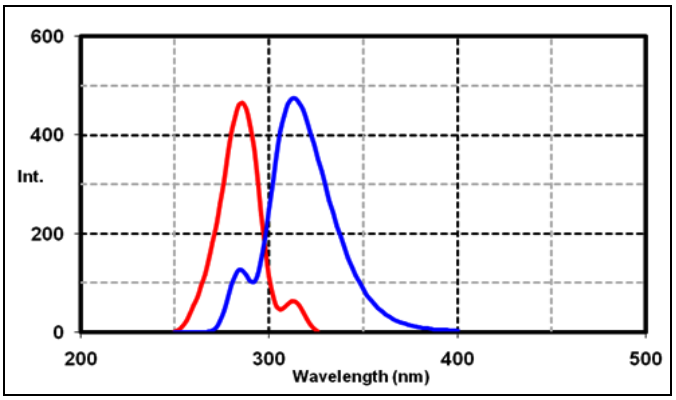

FIGURE 2: EXCITATION AND EMISSION SPECTRA OF TOLTERODINE (5 µg/ml) IN METHANOL USING 1 ml of PHOSPHATE BUFFER pH 6.

Optimization of Experimental Conditions:

(i) Effect of Diluting Solvents: The general procedure for the method was repeated using a fixed amount of TOL (50 µg) and different diluting solvents and found that; methanol is the best diluting solvent as shown in **figure 3**.

(ii) Effect of pH and Buffer: The general procedure for the method was repeated using a fixed amount of TOL (50 µg) and different buffers with different pH and found that; phosphate buffer pH 6 gives the best results as shown in **figure 4**.

(iii) Effect of Buffer Volume: The general procedure for the method was repeated using a fixed amount of TOL (50 µg) and different volumes of phosphate buffer pH 6 and found that; 1 ml gives the best results as shown in **figure 5**.

(iv) Effect of Time: The general procedure for the method was repeated using a fixed amount of TOL (50 µg) at different time interval and found that; it is stable at least for one hour as shown in **figure 6**.

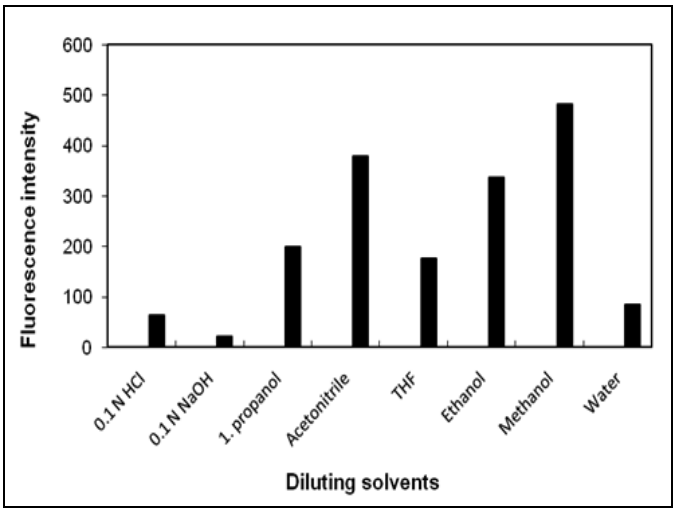

FIGURE (3): EFFECT OF DIFFERENT DILUTING SOLVENTS ON FLUORESCENCE INTENSITY OF TOLTERODINE (5 µg/ ml).

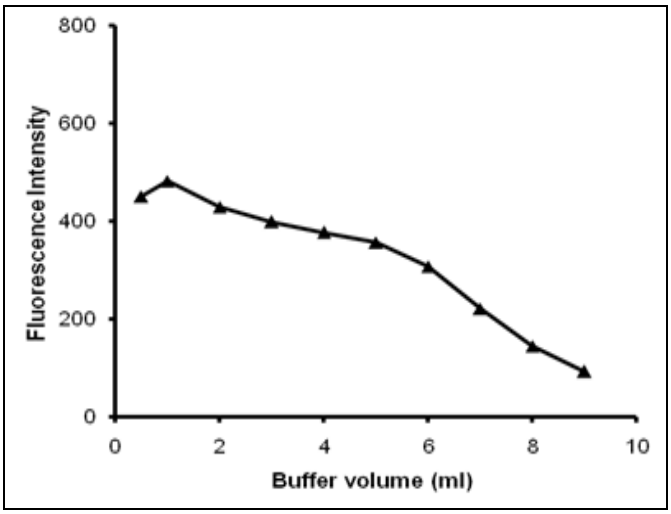

FIGURE (5): EFFECT OF VOLUME OF PHOSPHATE BUFFER PH 6 ON FLOURESCENCE INTENSITY OF TOLTERODINE (5 µg/ ml)

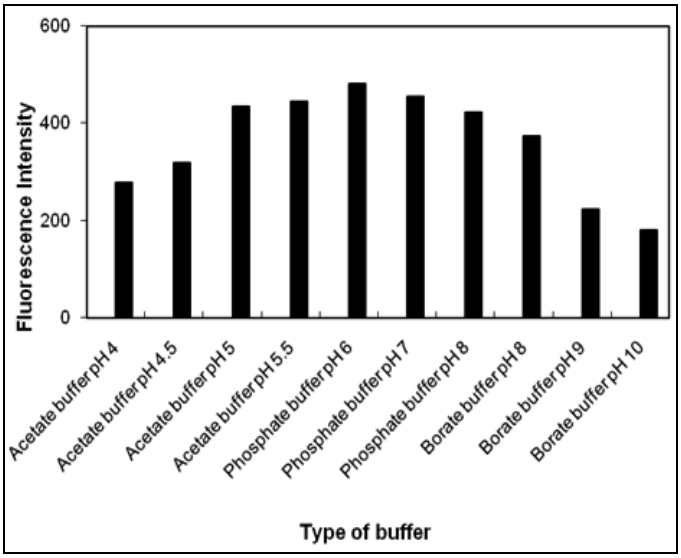

FIGURE 4: EFFECT OF DIFFERENT BUFFERS (1 ml) ON FLUORESCENCE INTENSITY OF TOLTERODINE (5 µg/ ml).

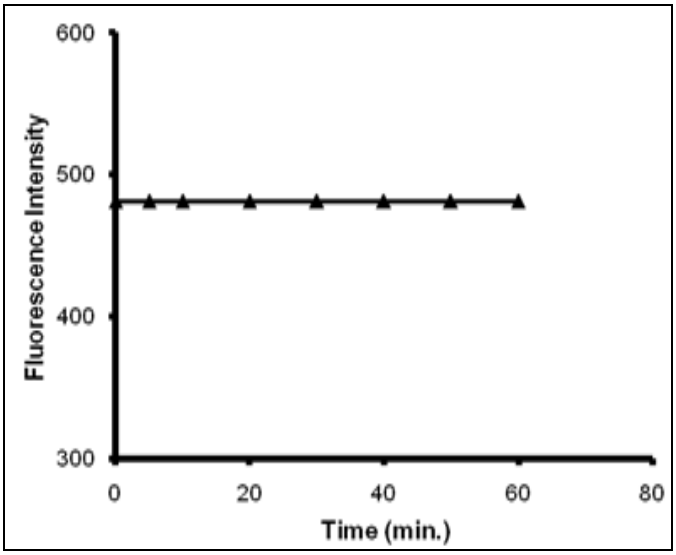

FIGURE (6): EFFECT OF TIME ON FLUORESCENCE INTENSITY OF TOLTERODINE (5 µg/ ml)

Validation of the Method:

(i) **Linearity:** Under the described experimental conditions, the calibration graph for the method was constructed by plotting fluorescence intensity versus concentration in µg/ml. The regression plot was found to be linear over the range of 0.5-8 µg/ml. The linear regression equation for the graph is:

$$\mathbf{FI = 94.80C + 0.354 \ (r^2 = 0.9998)}$$

Where **FI** is the fluorescence intensity, **C** is the drug concentration in µg ml^{-1} and $\mathbf{r^2}$ is the correlation coefficient.

Linearity range, regression equation, intercept, slope and correlation coefficient for the calibration data were presented in **table 1**.

TABLE 1: SPECTRAL DATA FOR DETERMINATION OF TOL BY THE PROPOSED METHOD:

Parameters	Proposed Method
$\lambda_{emission}$ (nm)	313
$\lambda_{excitation}$ (nm)	285
Linearity range (µgml^{-1})	0.5 — 8
LOD (µgml^{-1})	0.090
LOQ (µgml^{-1})	0.273
Regression equation*	
Slope (*b*)	94.80
Intercept (*a*)	0.354
Correlation Coefficient (r^2)	0.9998

* $y = a + bx$ where y is the fluorescence intensity and x is the concentration.

(ii) **Sensitivity:** The limit of detection (LOD) and the limit of quantitation (LOQ) were calculated according to ICH Q_2 Recommendation[20] from the following equations:

$$LOD = 3.3 \ S_a / slope$$

$$LOQ = 10 \ S_a / slope$$

Where S_a is the standard deviation of the intercept of regression line.

LOD was found to be 0.090 µg/ml, while LOQ was found to be 0.273 µg/ml. The small values of LOD and LOQ indicate good sensitivity.

(iii) **Accuracy and Precision:** Three replicate determinations of three different concentrations of TOL in pure form within linearity range were performed in the same day (intra-day) and in three successive days (inter-day). Accuracy as recovery percent (R%) and precision as percentage relative standard deviation (RSD%) were calculated and results are listed in **table 2**. The small value of RSD% indicates high precision of the method. Moreover, the good R% confirms excellent accuracy.

TABLE (2): INTRADAY AND INTERDAYS ACCURACY AND PRECISION FOR THE DETERMINATION OF TOL BY THE PROPOSED METHOD

Conc. µg.ml^{-1}	Intra-day			Inter-day		
	Found Conc. ± SD	Accuracy (R%)	Precision (RSD%)	Found Conc. ± SD	Accuracy (R%)	Precision (RSD%)
1	0.99 ± 0.008	99.44	0.798	1.00 ± 0.006	100.22	0.631
2	1.98 ± 0.024	99.23	1.215	1.98 ± 0.020	99.11	0.999
4	4.02 ± 0.033	100.40	0.033	4.02 ± 0.038	100.55	0.946

Pharmaceutical Applications: The proposed method was applied to the determination of the studied drug in its tablet preparation. The results were validated by comparison to a previously reported method [6]. No significant difference was found by applying t-test and F-test at 95% confidence level, indicating good accuracy and precision of the proposed method for the analysis of the studied drug in its pharmaceutical dosage form (**table 3**).

TABLE 3: DETERMINATION OF TOLTERODINE IN DETRUSITOL® TABLETS (2 mg) BY THE PROPOSED AND REPORTED METHODS

Parameters	Proposed Method	Reported method [6]
N*	5	7
$\bar{X}$	100.57	99.86
SD	0.76	0.64
RSD%	0.76	0.64
t*	1.69 (2.23)	——
F**	1.41 (6.16)	——

* No. of experimental. ** The values in the parenthesis are tabulated values of t and F at (p= 0.05).

CONCLUSION: The proposed method is simple, rapid and inexpensive. So, it is good alternative to the other few reported methods and to the high cost HPLC methods.

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