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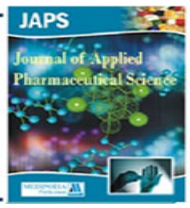

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Development and validation of spectrophotometric and spectrofluorimetric methods for simultaneous determination of Tofisopam

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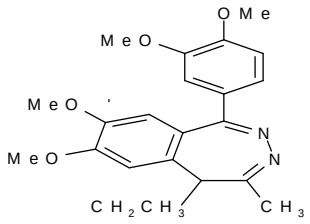
ABSTRACT

Two simple, rapid and accurate spectrophotometric and spectrofluorimetric methods developed for the determination of Tofisopam (TF) in pure form and in pharmaceutical formulation. The spectrophotometric method (A) is based on the reduction of ferric into ferrous in presence of 1, 10-phenanthroline to give an orange –red colored ferroin complex measured at 510 nm. Method (B) spectrofluorimetric method is based on the oxidative coupling reaction of TF with 3-methylbenzothiazolin-2-one hydrazone (MBTH) hydrochloride in presence of cerium (IV) ammonium sulfate in an acidic medium. The quenching effect of TF on the fluorescence of excess cerous ions is measured at the emission λ_{em} 345 nm with excitation λ_{ex} at 296 nm. The factors affecting the reactions were carefully studied and optimized. Beer's law is obeyed in the range of 2-12 µg ml^{-1} for both methods with the mean percentages recovery of 100.04 ± 0.445 and 99.29 ± 0.563 for method (A) and (B), respectively.
The two proposed methods were successfully applied for the determination of TF in Nodeprine tablets. Statistical comparison between the results obtained by these methods and that obtained by the official method for the determination of the drug was done, and it was found that there was no significant differences between them.

Keywords: Spectrophotometry; spectrofluorimetry, Tofisopam, O-phenanthroline, MBTH

INTRODUCTION



Structure of Tofisopam; Molecular formula = $C_{22}H_{26}N_2O_4$.HCl; Molecular weight = 382.46

TF is a 2,3-benzodiazepine related structurally to 1,4 benzodiazepines such as diazepam and sharing some of the same actions. It is reported however, to be largely lacking in the selective, anticonvulsant, and muscle relaxant properties of the conventional benzodiazepines. TF has been given orally in the short-term treatment of anxiety disorders. The R-(+)-isomer, dextrofisopam, is under investigation in the treatment of irritable bowel syndrome (Sweetman, 2009).

The determination of TF is described in JP (Japanese pharmacopoeia., 1997) by non aqueous titration method. It was also determined by several methods involving spectrofluorimetric method, where the fluorescence was measured at 488 nm (Kasa *et al*., 1989), HPLC methods using different kinds of columns and different mobile phases (Sajgo *et al.*, 1981- Patthy and Salat, 1981 - Gesztesi *et al*., 1981- Baek *et al*., 2002- Zsila *et al*., 1993 and Valentik *et al.*, 1999) and capillary electrophoresis, on an phenyl silica stationary phase and detecation at 220 nm (Cahours *et al.*, 1999). GC-NPD has been also reported for detection of TF (Toth *et al*., 2006), TF was also determined by polarographic method (Oelschlaeger *et al.*, 1985). The present study describes two simple, selective and economical spectrophotometric and spectrofluorimetric methods for the determination of the drug in its pure form and in pharmaceutical formulation.

EXPERIMENTAL

Instrumentation

- UV-Vis spectrophotometer (SHIMADZU 1601, Japan).
- Shimadzu RF – 1501 spectrofluorimeter with 1-cm quartz cells was used for all measurements.

Reference Sample

Tofisopam (TF) - Pure sample was kindly supplied by Acapi Pharmaceuticals, batch number 200706180184, (Cairo, Egypt) and assayed for its purity according to a pharmacopoeial method to contain 99.60 ± 0.300 %.

Pharmaceutical formulation

Nodeprine tablets- Manufactured by: Acapi Pharmaceuticals, (Cairo, Egypt), batch no. 21081 labeled to contain 50 mg of TF in each tablet.

Chemical and reagents

- O-phenanthroline-Fe (III) mixture (El-Shiekh *et al.*, 2007, Mallikarjun *et al.*, 2006) , prepared by dissolving 0.5 gm of O-phenanthroline monohydrate and 0.4 gm ammonium ferric sulfate in 5 ml 1 M hydrochloric acid, then diluted to 250 ml with water. This solution is stable for one month if stored in refrigerator.
- 3-Methyl benzothiazolin-2-one hydrazone hydrochloride (MBTH.HCl; Sigma Aldrich, St. Louis, USA) 0.2 % (w/v) freshly prepared in distilled water.
- Sulphuric acid (0.5M aqueous solution; Loba- Chime Industrial Co., India).
- Cerric ammonium sulphate (Merck, Munich, Germany), 0.5 % (w/v) in 0.5M sulphuric acid.
- Acetonitrile (Sigma Aldrich, St. Louis, USA).
- Methanol, ethanol and acetone (Adwic El- Nasr pharmaceutical Chemicals Co., Egypt).
- 1, 4-Dioxane (BDH Chemicals Ltd., England).

Standard solutions

TF stock standard solution

(1 mg/ml) in methanol for the two suggested methods. Prepared by dissolving 25 mg of TF in a 25-ml volumetric flask, then the volume was completed to the mark with methanol.

Working standard solution for method (A)

(0.2 mg/ml) in methanol. Prepared by transferring 20 ml of TF stock standard solution in a 100-ml volumetric flask, then the volume was completed to the mark with methanol.

Working standard solution for method (B)

(0.02 mg/ml) in methanol. Prepared by transferring 2 ml of TF stock standard solution in a 100-ml volumetric flask, then the volume was completed to the mark with methanol.

Procedures

Construction of the calibration graphs for method (A)

Aliquots (0.1, 0.2… 0.6ml) of TF were separately transferred from its working standard solution (0.2 mg/ml) into a series of 20- ml test tubes then 2 ml of phen – Fe (III) mixture and 3 ml acetate buffer pH 4 were added. The tubes were heated in a boiling water bath for 30 minutes then cooled, and transferred quantitatively into a series of 10-ml volumetric flasks and adjusted to volume with distilled water.

The absorbance spectra of the orange color were measured at 510 nm against a similarly prepared blank. Linear calibration curve was constructed relating the absorbance at 510 nm to the corresponding concentrations of TF and the corresponding regression equation was computed at the specified wavelength.

Construction of the calibration graph for method (B)

Into a series of 10-ml volumetric flasks, aliquots (1, 2…6 ml) of TF were separately transferred from its working standard solution (0.02 mg/ml) followed by 0.5 ml of 0.2 % w/v MBTH and 1 ml of 0.5% w/v of cerric ammonium sulphate. The solutions were kept at room temperature for 15 minutes then the volumes were completed with methanol.

The fluorescence intensity was measured at λ_{em} 345 nm with λ_{ex} 296 nm at room temperature (25° C) against blank similarly prepared. The calibration curve was constructed relating the fluorescence intensity difference (between blank and experiments) at λ_{em} 345 nm with λ_{ex} 296 to the corresponding concentrations of TF and the regression equation was computed.

Application of the proposed methods to the analysis of TF in pharmaceutical preparation

Five tablets were weighed accurately and powdered. An amount of powder equivalent to 25 mg of TF was accurately weighed into 25-ml measuring flask and extracted with 15-ml methanol in an ultrasonic bath for 30 minutes, diluted to volume with the same solvent and filtered. Suitable dilutions were made using methanol to prepare tablet solution containing 0.2 mg/ml and 0.02 mg/ml for method (A) and (B), respectively. 0.2 ml and 2 ml

of the solution were accurately transferred to a 10-ml volumetric flask and were diluted to volume with methanol for method (A) and (B), respectively. Then the procedures were completed as described under *Construction of the calibration graphs*. The concentration of TF was calculated by substituting in the corresponding regression equations.

RESULTS AND DISCUSSION

Methods Development

Spectrophotometric method (A)

O-phenanthroline-Fe (III): (Phen-Fe III) as a reagent was used for the determination of many pharmaceutical formulations and biological fluids. It is a mixture of o-phenanthroline monohydrate and ammonium ferric sulfate (P.B. Issopoulas; 1991). Phen-Fe (III) was used for the determination of ascorbic acid (Sultan *et al.*, 1994), Ritodrine (Abd El-Ghaffar *et al.*, 2008) antibiotics (Abd El-Sattar *et al.*, 2001) and acetaminophene (Carmona *et al.*, 1989).

The present work was based on the reducing action of TF on Fe (III) to Fe (II) in its complex with 1,10- phenanthroline to give the orange – red colored ferroin complex that exhibits an absorption maximum at 510 nm, (Figure 1).

Various parameters affecting the reaction process were studied, the reaction was found to be sensitive to pH, as evident from (Figure 2), pH (3.5- 4.5) are the best for developing maximum color intensity. Therefore pH 4 was prepared by using acetate buffer.

And it was found that 3- 4 ml of acetate buffer pH 4 were the optimum volumes for development of the color thus 3 ml was used, (Figure 3).

Also the volume of phen- Fe (III) mixture was studied. Figure (4) showed that, maximum color intensity was obtained at 510 nm by using 1.5-3 ml, thus 2 ml was adopted for the reaction.

At room temperature, low and unreliable results were obtained, however the color reaction is accelerated at elevated temperature. Different temperatures were tried from 60°C- 100°C using thermostatic water bath ; where the maximum color intensity was obtained by heating in a boiling water bath for 25-50 minutes, Figure (5). Therefore 30 minutes heating in boiling water bath were recommended.

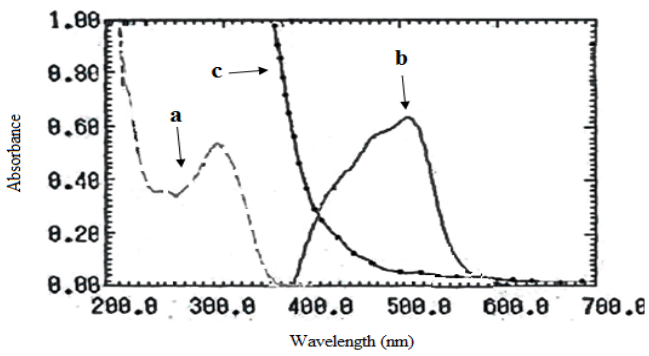


Fig.1: Zero Order Absorption Spectra of: a- TF in Methanol, 12µg/ml (----), b- TF – ferric phenanthroline Reaction Product, 6 µg/ml (─) & c- Blank Reagent (•─•)

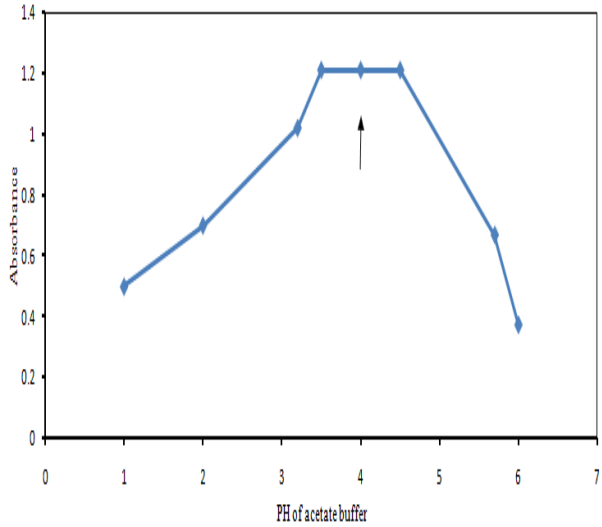


Fig. 2: Effect of Different pH values of acetate buffer on the Absorbance of TF – ferric phenanthroline Reaction Product at 510 nm (12 µg/ ml).

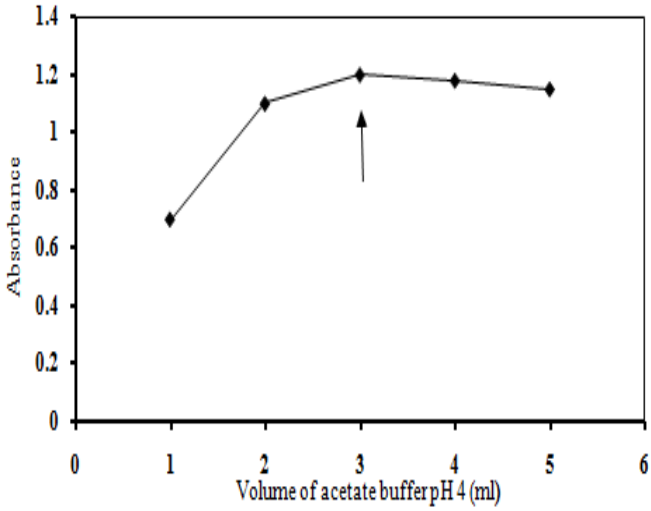


Fig. 3: Effect of the Volume of Acetate Buffer pH 4 on the Absorbance of TF – ferric phenanthroline Reaction Product (12 µg/ ml).

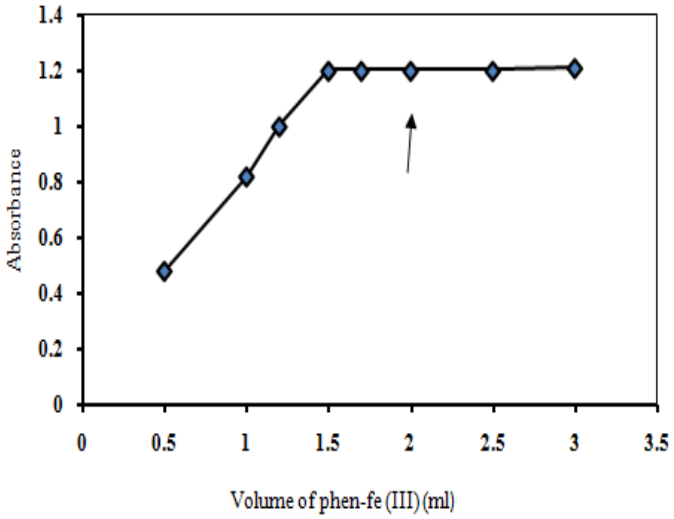


Fig. 4: Effect of the Volume of Phen-Fe (III) on the Absorbance of TF – ferric phenanthroline Reaction Product (12 µg/ ml).

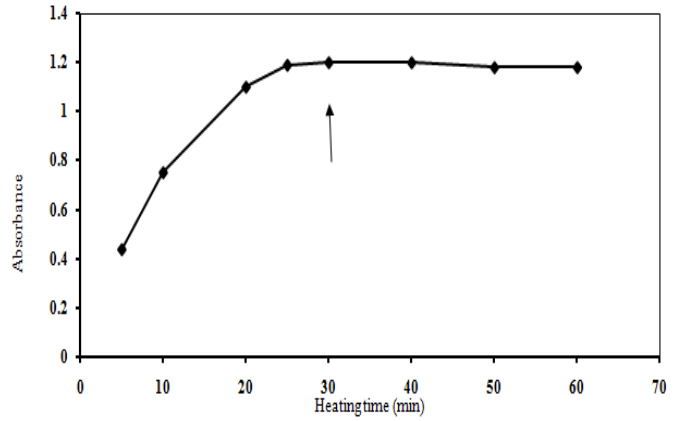


Fig. 5: Effect of Heating Time at 100 °C on the Absorbance of TF – ferric phenanthroline Reaction Product (12 µg/ ml).

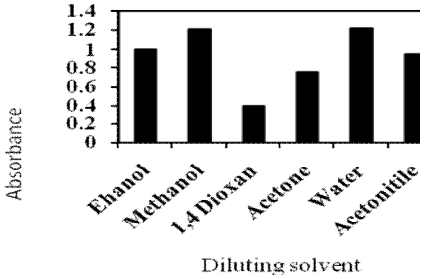


Fig. 6: Effect of Different Diluting Solvents on the Absorbance of TF – ferric phenanthroline Reaction Product (12 µg/ ml).

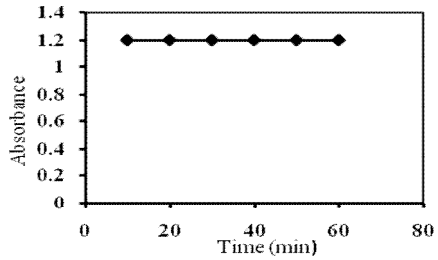


Fig. 7: Effect of Time on the stability of the Absorbance of TF – ferric phenanthroline Reaction Product (12 µg/ ml).

In order to select the most appropriate diluting solvent, the reaction was diluted using different solvents as absolute ethanol, methanol, 1, 4 dioxane, acetone, distilled water, and acetonitril. The maximum intensity was developed by using methanol or distilled water. Water was used as it is more safe and cheap, (Figure 6). It was found that the color intensity is stable and can be measured unaffected for further 60 minutes, (Figure 7).

Spectrofluorimetric method (B)

MBTH is a sensitive fluorogenic agent for the spectrofluorimetric determination of some nitrogen- containing drugs, including those with a heterocyclic ring (El-Gendy *et al.*, 2001), sulfa drugs (El- Kommos and Emara ., 1988), carbonyl derivatives (Rizk *et al.*, 2000) and some diuretics (Sastry *et al.*, 1988). Through its hydrazone grouping, MBTH reacts with TF in presence of cerium (IV) ammonium sulfate in an acidic medium to form an oxidative- coupling product. Ce (IV) as an oxidizing agent has been used for the determination of trimeprazine and trifluoperazine by measuring the fluorescence intensity of Ce (III) produced (Ruiz et ai., 1994; Hiremath *et al.*, 2008). Consequently upon reaction of Ce (IV) with MBTH strong fluorescence due to Ce (III) is obtained. The fluorescence intensity of the reaction product was measured at λ_{em} 345 nm with λ_{ex} 296 nm, (Figure 9). The reaction of cerium (IV) ammonium sulfate with MBTH (blank reagent) causes reduction of the Ce (IV) to Ce (III), which produces high relative fluorescence intensity. Upon the addition of TF, the fluorescence intensity of the solution decreased significantly and the magnitude of the decrease was proportional to the concentration of TF, (Figure 10).

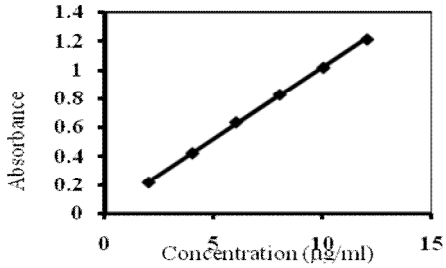


Fig. 8: Linearity of the Absorbance of TF – ferric phenanthroline Reaction Product to the Corresponding Concentrations of TF (2-12 µg/ml) at 510nm.

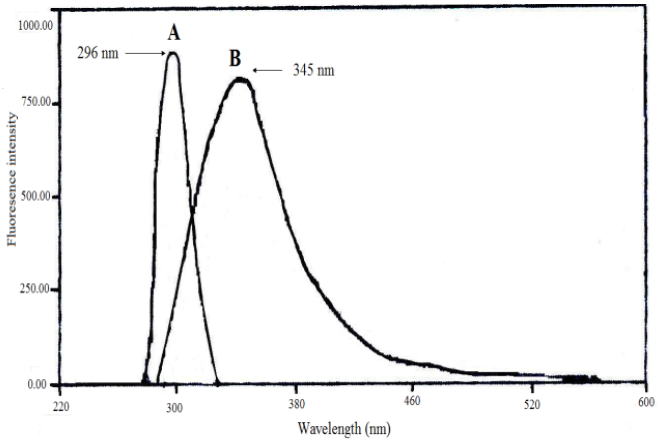


Fig. 9: Excitation (A) and Emission (B) Spectra of the Blank Reagent.

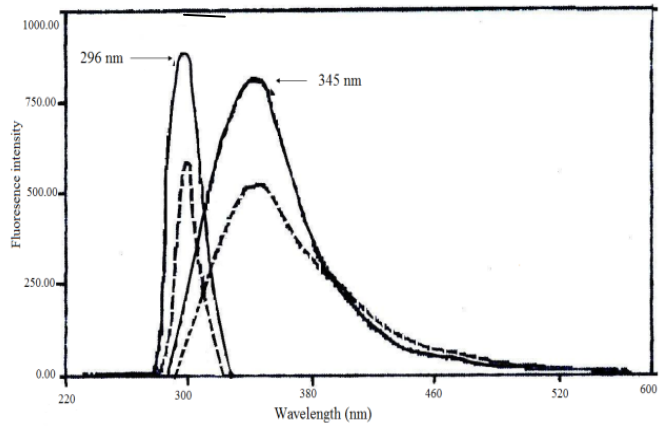


Fig. 10: • (____) Excitation (A) and Emission (B) Spectra of the Blank Reagent • (- - - -) The Quenching Effect of TF (6 µg/ml) on the Blank Reagent.

The quenching of Ce (III) fluorescence was observed may be either due to electrophilic substitution reaction or chelation of Ce (III) ions with a consequent decrease in fluorescence intensity. Various parameters affecting the reaction process were studied, the reaction was found to be sensitive to volume of MBTH (0.2% w/v), 0.5 ml was found to be sufficient to produce maximum fluorescence intensity difference as evident in Figure (11). Also the volume of cerric ammonium sulfate (0.5% w/v) was studied and 1 ml was the best for the reaction condition as in (Figure 12).

The optimum reaction time was determined at room temperature (25° C) and maximum fluorescence intensity difference was attained after 15 min which remained stable for 30 min as shown in Figure (13).

In order to select the most appropriate diluting solvent, the reaction was carried out using different solvents as distilled water, methanol, ethanol, acetone, 0.5M sulfuric acid and acetonitrile. Methanol was found to be ideal solvent for MBTH as shown in Figure (14).

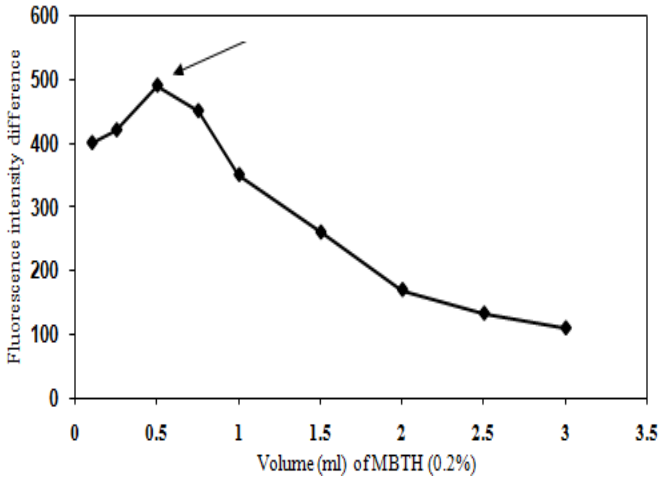


Fig. 11: Effect of Different Volumes of MBTH (0.2 % w/v) on the Fluorescence Intensity Difference of TF (6µg/ ml) – MBTH Reaction Product .

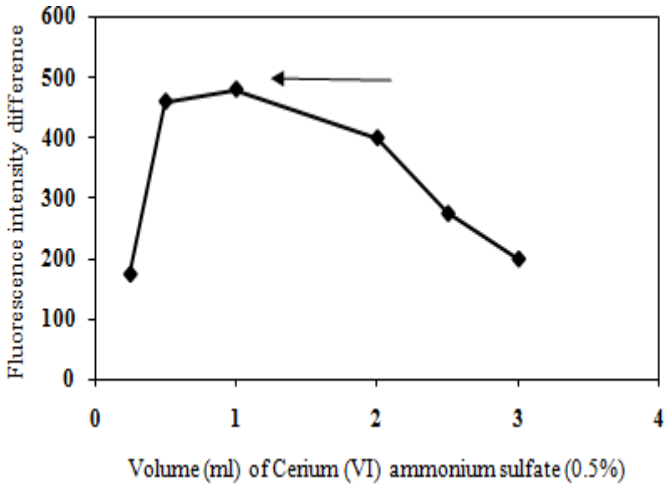


Fig. 12: Effect of Different Volumes of Cerium (IV) Ammonium Sulfate (0.5 % w/v) on the Fluorescence Intensity Difference of the TF (6µg/ ml) – MBTH Reaction Product .

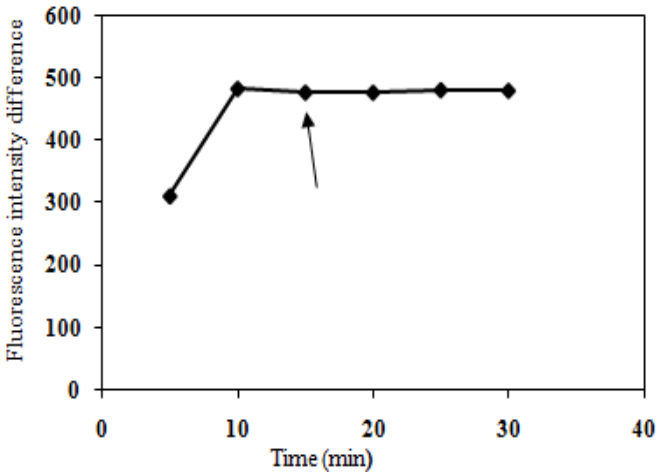


Fig. 13: Effect of Time on the Fluorescence Intensity Difference of the TF (6µg/ ml) – MBTH Reaction Product.

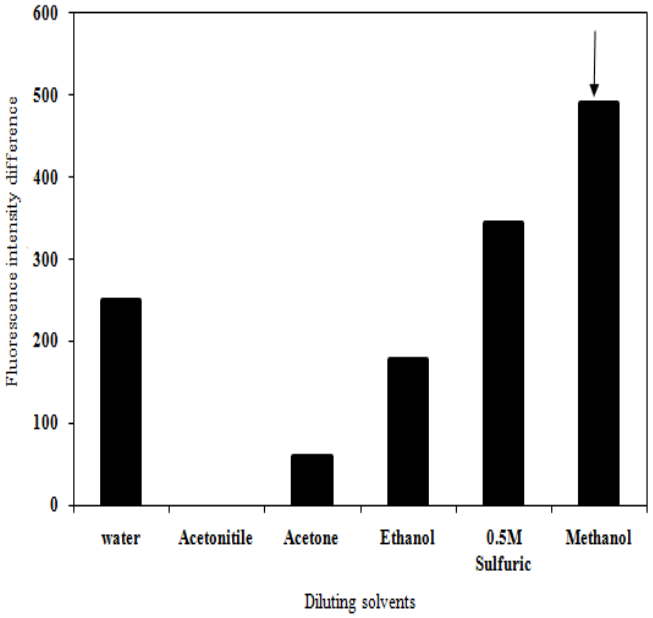


Fig. 14: Effect of Different Diluting Solvents on the Fluorescence Intensity Difference of the TF(6µg/ ml) – MBTH Reaction Product.

Methods Validation

Linearity

Under the specified optimum reaction conditions, the linearity was checked and the calibration curves were constructed between the absorbance and corresponding concentration at the selected wavelength for method (A) and between the fluorescence differences versus corresponding concentration of TF for method (B) . The proposed methods were found to be valid in the range of 2- 12 µg/ml as shown in Figures (8, 15). The regression equations were computed and found to be:

$A_{\text{method A}} = 0.0997\ C + 0.0259$ $\quad r = 0.9995$

$Y_{\text{method B}} = 61.32C + 107.53$ $\quad r = 0.9995$

Where, A = the absorbance at 510 nm, Y = fluorescence difference, C = Concentration of TF in µg/ml and r = Correlation coefficients.

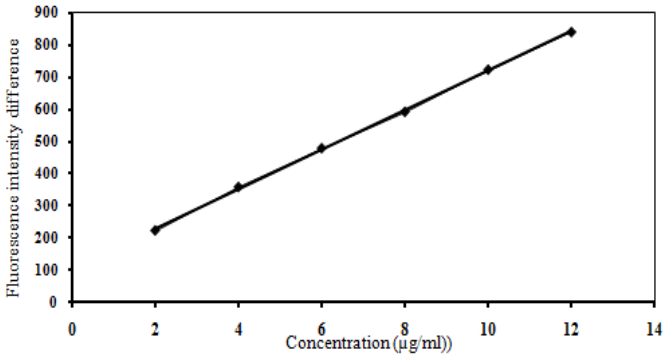


Fig. 15: Linearity of the Fluorescence Intensity Difference of TF at λ_{em} 345 nm with λ_{ex} 296 nm to the Corresponding Concentrations of TF (2- 12µg/ml).

Accuracy

The proposed methods were successfully applied for the determination of the drug in pure powdered form with mean percentage recoveries of 100.04 ± 0.445% at 510 nm for method (A) and 99.29 ± 0.563% for method (B), (Table 1).

Precision (Repeatability and Intermediate precision)

The intra-day and inter-day precision were evaluated by assaying three freshly prepared solutions of the drug in triplicate on the same day and on three successive days, respectively at concentrations 4, 8, 10 µg/ml for the two proposed methods , the results in Table (1) showed good precision..

Table . 1: Results of assay validation obtained by applying the proposed spectrophotometric [Phen-Fe (III)] and spectrofluorimetric (MBTH) methods for the determination of TF in its pure powdered form.

Parameters	Spectrophotometric method [Phen-Fe(III)]	Spectrofluorimetric method(MBTH)
Validation of response		
Linearity range (µg/ml)	2-12	2-12
LOQ (µg/ml)	1.035	0.219
LOD (µg/ml)	0.034	0.072
Accuracy *		
Mean ± R.S.D%	100.04 ± 0.445	99.29 ± 0.563
Precision		
Repeatability **± R.S.D%	99.24 ± 0.222	99.23 ± 0.842
Intermediate precision ** ± R.S.D%	99.29 ± 0.595	99.27 ± 0.145
Ruggedness*** ± R.S.D%	99.78 ± 0.567	100.82 ± 0.873
Robustness*** ± R.S.D%	100.43 ± 0. 653	100.32 ± 0.430
Validation of regression equation		
Slope	0.0997	61.329
SE of slope	1.0830×10^{-3}	0.7053
Confidence limit of the slope ****	0.0967 / 0.1027	59.370 / 63.287
Intercept	0.0259	107.533
SE of intercept	8.4370×10^{-3}	5.4933
Confidence limit of the intercept ****	0.0025 / 0.0494	92.281 /122.785
Correlation coefficient	0.9995	0.9995
SE of estimation	9.0630×10^{-3}	5.9008

* n = 6. ** n = 3×3. *** n = 3. **** 95% confidence limit.

Table . 2: Quantitative determination of TF in pharmaceutical formulation by the proposed spectrophotometric [Phen-Fe(III)] and spectrofluorimetric (MBTH) methods and the result of application of standard addition technique.

Pharmaceutical formulation	Proposed methods	Found% * of claimed amount ± R.S.D	Standard added (µg/ml)	Recovery% *of standard added
Nodeprine tablets (50mg TF /tab) B.N. 21081	**Spectrophotometric method [Phen-Fe(III)]**	99.37 ± 0.732%	2	98.50
			4	98.70
			8	99.91
	Mean ± R.S.D	99.04 ± 0.763		
	Spectrofluorimetric method(MBTH)	100.10 ± 0.539 %	2	99.00
			4	99.50
			8	98.10
	Mean ± R.S.D	98.87 ± 0.709		

*Average of three different determinations. Claimed amount (4µg/ml).

Table. 3: Statistical comparison between results obtained by applying the proposed spectrophotometric [Phen-Fe(III)] and spectrofluorimetric (MBTH) methods and the official method for determination of TF in pure powdered form.

Item	Spectrophotometric method [Phen-Fe(III)]	Spectrofluorimetric method (MBTH)	Official method*[8]
Mean	100.04	99.29	99.60
S.D.	0.445	0.559	0.300
R.S.D.%	0.445	0.563	0.301
SE	0.182	0.228	0.134
Variance	0.198	0.312	0.090
n	6	6	5
t-test **	1.872 (2.262)	1.107 (2.262)	
F-test **	2.200 (6.26)	3.467(6.26)	

* Non aqueous titration (using glacial acetic acid as a solvent and 0.1M perchloric acid as a titrant, the end point was detected potentiometrically).

Robustness and Ruggedness

To study the method ruggedness, three different concentrations (4, 8, 10 µg/ml) solution of TF were analyzed in two different laboratories using two different instrument. The result in Table (1) proved the stability of the methods upon change the instrument. As for the robustness, determining (4, 8, 10 µg/ml) solution of TF using 3 ml of the phen Fe (III) instead of 2 ml was studied for method (A), while in case of method (B) at 30º C instead of room temperature (25ºC) was studied, the methods demonstrated sufficient stability, (Table 1).

Assay of pharmaceutical formulation

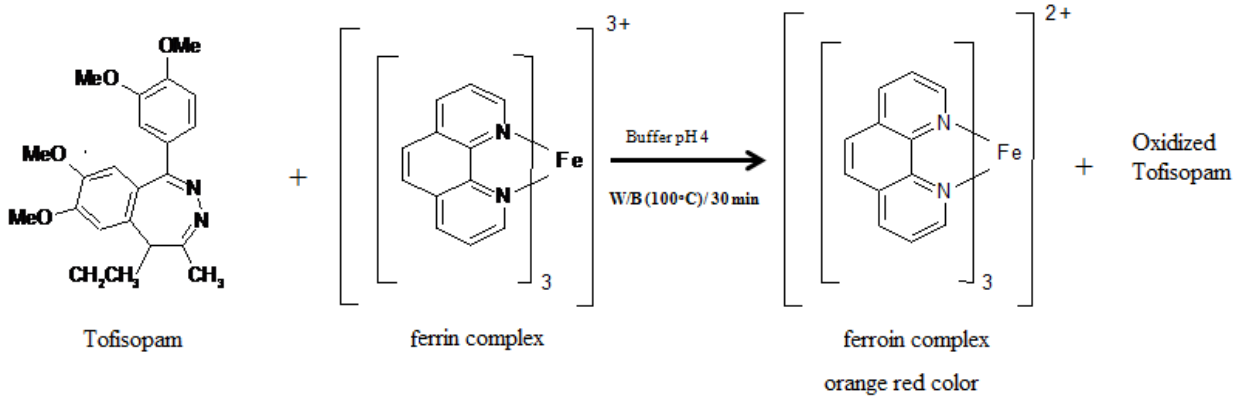
The proposed methods have been successfully applied to assay TF in Nodeprine tablets. The validity of the proposed methods was further assessed by applying the standard addition technique for the analysis of Nodeprine tablet as shown in Table (2).

Comparison with the official method

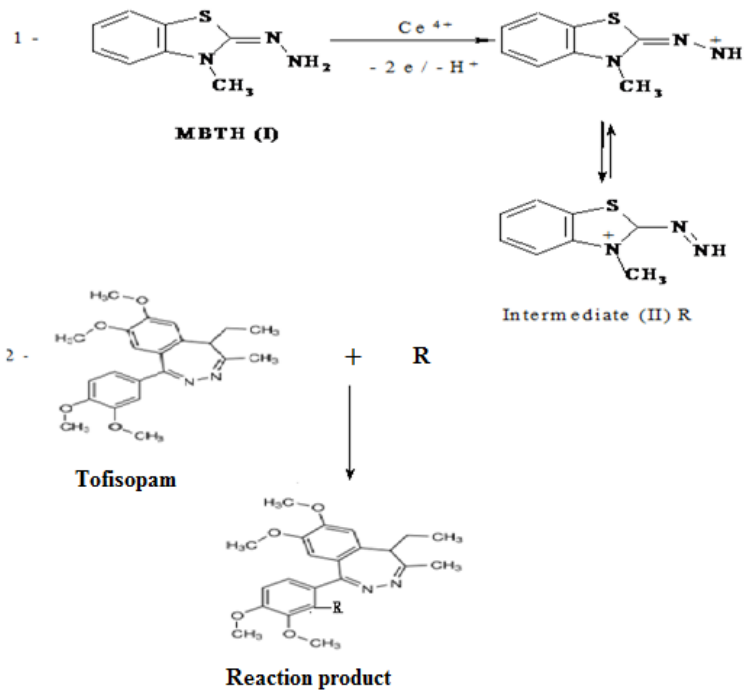
The results obtained by applying the proposed methods for the analysis of the studied drug in pure form were statistically compared with those obtained by applying the official method for TF. The values of the calculated t and F are less than the tabulated ones which reveals that there is no significant difference with respect to accuracy and precision (Gardiner., 1997) as shown in Table (3).

CONCLUSION

From the data obtained it is proved that the proposed spectrophotometric {phen-Fe (III)} and spectrofluorimetric (MBTH) methods are simple, accurate, precise, and could be applied in quality control laboratories for quantitative determination of the drug in pure powdered form and in pharmaceutical formulation



Scheme . 1: The Suggested Mechanism for the Reaction of Tofisopam with Fe- phenthanthroline mixture.



Scheme. 2: The Suggested Mechanism for the Reaction of Tofisopam with MBTH.

REFERENCES

Abd El-Ghaffar M., El-Wass eef D. and El-Ashry S. Spectrophotometric methods for determination of ritodrine in bulk and in pharmaceutical dosage forms; J. Food and Drug Analysis.2008; 16 (2): 26-35.

Abd El-Sattar O., El-Abasawy N., Abdel-Razeq S., Ismail M. and Rashed N. Colorimetric determination of six cephalosporins of second and third generation in the range of micromolar concentrations; J. Saudi Pharm.2001; 9 (3) : 186- 190.

Beak S.K., Choi S.J., Kim J.S., Park E.J., Sohn D.H., Lee H.Y. and Lee H.S. Analysis of tofisopam in human serum by column-switching semi-micro high-performance liquid chromatography and evaluation of tofisopam bioequivalence; Biomedical chromatography. 2002; 16 (4): 277-281.

Cahours X., Morin P., and Dreux M. Determination of tofisopam in serum by high-performance liquid chromatography; J.Chromatogr.A .1999; 845 (1-2): 203-216.

Carmona M., Silva M. and Perez-bendito D. A sensitive spectrophotometric determination of acetaminophen; Anal. Chem. Acta.1989; 218 (2): 313-322.

El-Gendy A. E. , El-Bardicy M. G. , Loutfy H. M. and El-Tarras M. F. Stability indicating method for the determination of nizatidine using 3-methyl-2-benzothiazolinone hydrazone. Spectro. Lett. 2001; 34 (2): 221-234.

El-Kommos M. E., Emara K. M. Application of 3-methylbenzothiazolin-2-one hydrazone as achromogenic reagent for the spectrophotometric determination of certain sulpha drugs; J. Analyst.1988; 113: 133- 137.

El-Shiekh R., Amin A., Zahran F. and Gouda A. Spectrophotometric determination of pipazethate hydrochloride in pure form and in pharmaceutical formulation; J. AOAC Inter.2007 ; 90 (3): 686-692.

Gardiner W.P. Statical Analysis Methods for Chemists: A Software-Based Approach. The Royal Society of Chemistry, UK, (1997) pp. 42-72, 330-333.

Gesztesi A., Sido-Lenarth T., Sajjgo M., and Fazekas A. Clinical pharmacokinetic profile of tofisopam ;Zentralbl Pharm., pharmakother-Laboratoriumsdiagn 1981; 120(6): 642-647

Hiremath B., Mathada B.H., Ayaswamy M. A validated spectrofluorimetric method for determination of trimeprazine and trifluoperazine via oxidation with ce (iv); Acta Pharm. 2008; 58: 275-285.

Issopoulas P.B. Sensetive spectrophotometric determination of micro amount of d-penicilliamine using an in direct redox technique; Acta. Pharm. Hung. 1991; 61: 205-210.

Japanese Pharmacopoeia: Official Monographs.13 th ed. (1996) 679-980.

Kasa L., Hornyak L., Korosi J., and Hamori T. Spectrofluorimetric method for the determination of 1-(3,4-dimethoxyphenyl)-5-ethyl-7,8-dimethoxy-4-methyl-5H-2,3-benzodiazepine (tofisopam); Anal. Chem. Acta.1989; 227(1): 315-318.

Mallikarjun K., Moses-Babu J., Sudhakar P., Sharma MSP and Sudershan G. Spectrophotometric and spectrofluorimetric methods for determination of racecadotril. Pharmazie, 2006; 61(12): 994-998.

Oelschlaeger H., Volki J., Bresch C. Analyses of drugs by polarographic methods. The polarographic behaviour of tofisopam; Arch. Pharm. (Weinheim, Ger) .1985; 318 (3): 271-276.

Patthy M., and Salat J. High-performance liquid-chromatographic determination of grandaxin [tofisopam] (a benzo-2,3-diazepine) and its trace impurities; J-Chromatogr.1981; 210 (1): 159-162.

Rizk M., Belal F., Ibrahim F., Ahmed S. M., El- Enany N. M. Spectrofluorimetric methods of stability-indicating assay of certain drugs affecting cardiovascular system;J. Sci. Pharm. 2000; 68:173- 188.

Ruiz T.P., Lozanzo C.M., Tomas V., and Cardona S.D. Simple flourimetric determination of certain antiviral drug via their oxidation with ce (iv); Talanta. 1994; 40 (9): 1361-1365.

Sajgo M., gesztesi A., and Sido T., Determination of tofisopam in serum by high-performance liquid chromatography;J. Chromatography, Biomed. appl .1981; 11 (2): 303-307.

Sastry C. S. P., Prasad T. N. V., Sastry B. S., and Rao E. V. *Spectrophotometric* methods for determination of some diuretics *using 3-methyl-2-benzothiazoline hydrazone*;J. Analyst. 1988; 13: 255- 258.

Sultan S.M., Abdennabi A.M. and Suliman F.E.O. Improvement of an old spectrophotometric method for the microdetermination of ascorbic acid by the use of a micellar medium; Talanta. 1994; 41 (1): 125-130.

Sweetman S.C. Martindale: The Complete Drug Reference. 36 th ed. The Pharmaceutical Press, London, UK, (2009) 1033.

Toth M., Bereczki A., Drabant S., Nemes K.B., Varga B., grezal G., Tomlo J., Lakner G., and Klebovich I. Gas chromatography nitrogen phosphorus detection (GC-NPD) assay of tofisopam in human plasma for pharmacokinetic evaluation; J. of Pharm. Biomed. Anal.2006; 41(4): 1354-1359.

Valentik M., Karaffa E., and Ladanyi L. Investigation of potential impurities in tofisopam by high-performance liquid chromatography;Acta. Pharm. Hung.1993 63 (2) 57-65.

Zsila F., Gergely A., HorvathP., and Szasz G. Separation and identification of tofisopam stereoisomers by hyphenated HPLC-CD technique; J. of Liquid Chromatography and Related Technologies.1999; 22 (5): 713-719.