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Original Research Article

Development of Two Charge-Transfer Complex Spectrophotometric Methods for Determination of Tofisopam in Tablet Dosage Form

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Abstract

Purpose: *To develop an easy, fast and sensible spectrophotometric method for determination of tofisopam in tablet dosage form.*
Methods: *Tofisopam as n-electron donor is react with two π-acceptors namely: chloranilic acid (ChA), and 7,7,8,8 tetracyanoquinodimethane (TCNQ) to form charge-transfer complexes. The obtained complexes were tested spectrophotometrically at 520 and 824 nm for ChA and TCNQ, respectively. The optimal conditions affecting the reaction status were surveyed and optimized, and the results compared with Japanese Pharmacopeia method.*
Results: *The calibration curve were obeyed Beer`s low in the ranges 25 – 125 and 30 – 150 µg/mL for ChA and TCNQ, respectively. The lower limit of detection was 8.0 and 10.0 µg/m for ChA and TCNQ, respectively. The slope and intercept of the calibration graphs were 0.0025 and 0.011, and 0.0115 and -0.237 for ChA and TCNQ, respectively*
Conclusion: *The proposed methods have successfully been applied to determination of tofisopam with good accuracy and precision. The methods are accurate as the Japanese pharmacopeial method amd may be applied for routine analysis in quality control laboratories.*

Keywords: *Charge-transfer complex, Tofisopam, Chloranilic acid, Tetracyanoquinodimethane, Spectrophotometry*

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INTRODUCTION

Tofisopam is a 1, 2-benzodiazepine and is analogous with other anxiolytic benzodiazepines which are generally 1,4- or 1,5-substituted. However, tofisopam does not have anticonvulsant, calming [1], skeletal reduce tension, motor skill-damaging or amnestic [2] features (Figure 1) [3]. Tofisopam is used for the therapy of worry and alcohol retraction, and is prescribed in a dose of 50 - 300 mg daily, separated into three doses. Tofisopam is not reported to cause dependence to the same extent as other benzodiazepines, but is advised to be prescribed for a maximum of 12 weeks.

Tofisopam is a PDE10A inhibitor, is said to be a beneficial therapy for mental disorder (4). The pharmacological effectiveness of tofisopam (TOF) and its inherent toxicity have encouraged efforts aimed at the development of different assay methods for the drug.

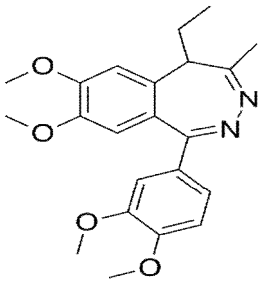

Figure 1: Chemical structure of tofisopam

Spectrophotometry [5,6], spectrofluorometry [5,7], high performance liquid chromatography (HPLC) using either reverse-phase chromatography [8-10] or enantiomeric separation [11,12], gas chromatography (GC) [13,14], and super critical chromatography [15] have been cited in the literature for the determination of tofisopam in its dosage form. Although, most of these methods are sensitive, however, they include much time, derivatization procedure, high price that are not obtainable in most quality control laboratories.

However, only two spectrophotometric methods have been reported [5,6]. The first method is based on reduction of ferric into ferrous in the presence of 1,10-phenanthroline to give an orange-red colored ferroin complex that absorbs at 510 nm [5], while the second method is based on derivative technique [6].

Charge-transfer complexes [16,17] are based on the interaction between electron donors and acceptors, which are characterized by absorption of radiation in ultra-violet and visible regions (200 - 800 nm). Compounds of n-electron donor react rapidly with π- acceptors and lead to form a fast complex at normal conditions. Π-Acceptors are used for the development and establishment of spectrophotometric methods for the determination of–n-electron donor compounds, especially biologically active pharmaceutical compounds. The aim of the present study was to investigate the charge transfer reaction between n-donor (tofisopam) with π- acceptor (ChA and TCNQ) to form a charge-transfer complex for analysis of tofisopam.

EXPERIMENTAL

Apparatus

A Shimadzu UV-1800 double-beam ultraviolet-visible spectrophotometer with 1-cm quartz cells was used.

Materials and reagents

Chloranilic acid (ChA), purchased from Merck, Darmstadt, Munich, Germany. A 1 mg/mL solution of chloranilic acid was prepared in acetonitrile. 7, 7, 8, 8-Tetracyanoquinodimethane was obtained from Aldrich Chemical Co., Milwaukee, USA; its solution (1 mg/mL) was prepared in acetonitrile. All reagent solutions were stable for at least 1 week when kept in a refrigerator. Pure tofisopam (≥ 98 %) was purchased from Sigma (St Louis, Mo, USA). Stock solution of tofisopam (500 µg/mL) was prepared by dissolving the exact amount of tofisopam in acetonitrile. Suitable working solutions were prepared by suitable dilution of the stock solution in acetonitrile. Acetonitrile was of analytical reagent grade. Nodeprine tablets was obtained from Acapi Pharmaceuticals, Cairo, Egypt, and each contained 50 mg of tofisopam.

Determination of tofisopam in its dosage form

Ten tablets were weighed accurately and ground well. A quantity of the powder equivalent to 50 mg of TOF was transferred into 100 mL measuring flask and dissolved in acetonitrile in an ultrasonic bath for about 40 min. The solution was filtered through Whatman filter paper no. 41, then the filtrate was transfer into a 100 mL volumetric flask and diluted to the mark with acetonitrile. Another appropriate aliquot of this solution was transferred into 10 mL volumetric flask, then the unknown concentration of the drug was determined as recommended in the next section.

Procedure

An appropriate amount of the standard solution of tofisopam covering the calibration graph was transferred into a 10 ml calibrated flasks, then one mL of ChA reagent was added. Complete the flask with acetonitrile and read the absorbance at 520 nm. In case of TCNQ, An appropriate amount of sample solution of tofisopam covering the calibration graph was transfer into test tube, 1 mL of TCNQ reagent was added, then heated at 70 ^{o}C for 20 min in water bath. The resulting solution was cool down, then transfer into 10-ml measuring flask and completed to the mark with acetonitrile. The absorbance of the outcome solutions was measured at the 842 nm. The calibration curve for each reagent was prepared by plotting absorbance versus concentration of tofisopam.

Validation of the proposed method

Under the optimum reaction situations, the calibration curves for tofisopam were developed by prepared an appropriate concentration range of the drug with each reagent. Each concentration was tested five times and the average absorbance was estimated, then plotted against concentration. Regression equations of the obtainable data were extracted from the calibration curve.

RESULTS

Tofisopam is a nitrogenous compound that act as n-donor which react with π-acceptors e.g. chloranilic acid (ChA) and 7, 7, 8, 8 tetracyanoquinodimethane (TCNQ) to form charge transfer complex. The resulting complexes exhibit an absorption maximum at 520 nm with ChA, instantaneously while the other complex exhibit a maximum absorbance at 841 nm (TCNQ) after heating at 70 ^{o}C for about 20 min. In case of ChA, the purple color instantaneously is formed at room temperature. On the other hand, the producing complex with TCNQ reagent exhibit a blue color was formed when heated at 70 ^{o}C for about 20 min. One ml of TTCNQ solution was found to be suitable for production of maximum absorbance. The intensity of the product color remains stable for about 1 h.

Effect of reagent concentration

Varying reagent concentrations (1 mg/mL, acceptors) were added to a constant concentration of tofisopam, but it was noticed that 1.0 mL each of ChA and TCNQ, respectively, was adequate for the production of maximum absorbance. Larger amounts of the reagents did not influence absorbance. Figure 2 shows the absorption curves of the drug with ChA and TCNQ reagents. As a result, 1 ml of reagent was used in all the tests.

Effect of reaction time and temperature

The optimum reaction time was monitored at by measuring the absorbance at 520 nm for ChA at room temperature (25 ^{o}C). Color was developed instantaneously and the intensity of the resulting color did not change over time. The color produced was stable for about 1 h. On the other hand, TCNQ formed an intense chromogen with tofisopam when heated at 70 ^{o}C for 20 min. However, at room temperature, only a faint color appeared and the absorbance of the color increased with increase in time. The development of maximum color intensity was achieved when heated at 70 ^{o}C for about 20 min.

Stoichiometry of the reaction

To determine the molar ratio between the drug (n-donor) and the reagent (π- acceptors), Job's method [18] of continuous variation was employed. Two master equimolar (2.83×10^{-4} M) solutions of TOF and ChA or TTCNQ were prepared. Different series of the master solutions of TOF and ChA as well as TOF and TCNQ, were made up in varying ratios (1:9, 2:8, 3:7, etc… and 9:1). The solutions were further measured as described above. The molar ratio of the reactants (drug: reagent) was determined by continuous variation method (Job's method) [18] to a form charge-transfer complex. As shown in Fig 3, a 1:1 ratio of the drug with ChA or TCNQ obtained.

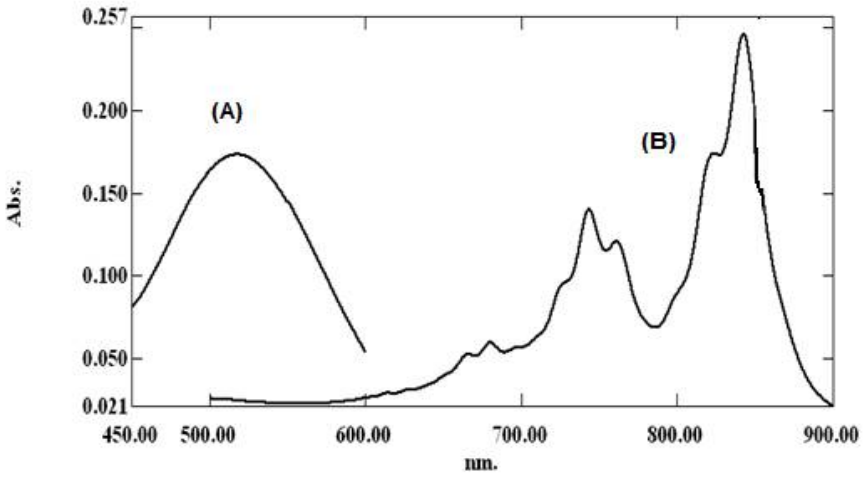

Figure 2: Absorption spectrum of tofisopam (50 µg/ml) with (A) ChA and (B) TCNQ reagents as π-acceptor

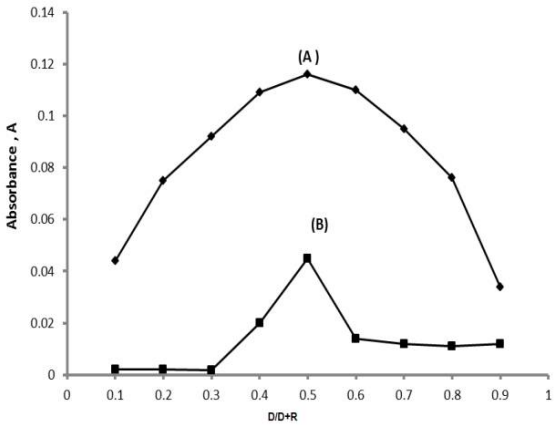

Figure 3: Continuous variation plot of tofisopam: (A) TOF: ChA and (B) TOF: TCNQ

Effect of diluting solvent

The charge transfer reaction was carried out in different solvents, including acetonitrile, acetone, ethanol, water, DMF and DMSO, in order to optimize the most suitable medium for the reaction. Acetonitrile proved to be the most suitable dilution solvent because it afforded superior solvating power for ChA and TCNQ reagents and gave high absorbance intensity. Acetonitrile was considered as an ideal solvent for the charge transfer reaction offering maximum sensitivity, due to its high dielectric constant which raised maximum yield of radical anions, and high solvation power for the acceptors [19].

Validity of the proposed method

Limit of quantification (LOQ) and of detection (LOD)

A calibration graph was linear over the concentration range of 25 – 120 ppm and 30 – 150 ppm for ChA and TCNQ, respectively. Lower limit of quantification (LOQ) and lower limit of detection (LOD) are presented in Table 1. The least square equation extracted from the calibration graph is presented in the following equation: $y = ax \pm b$, where y is the absorbance, a slope, x concentration, the exact equation is y =0.0025x+ 0.0115 and y =0.011x - 0.12373) for ChA and TCNQ, respectively. where y, a, x and r are the absorbance, x: concentration of TOF and a: is the slope , b : is the intercept and r is correlation coefficient (0.998 and 0.997 for ChA and TCNQ), respectively. The lower limit of detection (LOD) and quantification (LOQ) were estimated (21) using the formula: LOD or LOQ = K. SD. b/a where K= 3.3 for LOD and 10 for LOQ, SD b is the standard deviation of the intercept, and a is the slope. The lower limit of quantification was found to be 25.0 and 30.0 ppm while the lower limit of detection was 8.0 and 10.0 ppm for ChA and TCNQ respectively. The correlation coefficient was 0.998 and 0.997 for ChA and TCNQ, respectively.

Table 1*:* Quantification parameters for the analysis of tofisopam by the proposed method

Parameter	ChA	TCNQ
Beer`s law limits, µg/mL	25-125	30-150
Slope (*b*)	0.0025	0.011
Intercept (*a*)	0.0115	-0.2373
Correlation coefficient, (r^2)	0.998	0.997
Lower limit of quantification (LOQ, µg/mL)	25.0	30.0
Lower limit of detection (LOD, µg/mL)	8.0	10.0
Standard deviation of residual XY	0.0096	0.064

** A = a× ± b, where a = slope, b= intercept and × = concentration µg/ mL*

Interferences

In order to investigate the effect of commonly encountered compounds that are present in tofisopam dosage form, interference studies were carried out. Tablet excipients such as starch, lactose and glucose had a negligible effect on the determination of tofisopam. Thus, the proposed method can be applied for determination of tofisopam in the dosage form without interferences from the excipients. The results in Table 3, show a mean range of 96.8 - 99.6 and 96.6 - 98.6 % for ChA and TCNQ, respectively, indicating that there was no interference from the excipients present in the dosage form.

Precision and accuracy of the method

Intra-day and inter-day accuracy and precision of the developed method were investigated The accuracy and precision were and carried out by the analysis of tofisopam using six replicates during the day and on different days in the limit of quantification range. Precision and accuracy of the proposed method are expressed as RSD (%) and recovery (%), respectively. The results in Table 2 are within the acceptance range. Intra-day and inter-day precision were 2.7 - 3.0 and 2.6 - 2.9 %, and 2.7 - 3.2 and 2.5 - 3.0 % for TOF-ChA and TOF-TCNQ, respectively. On the other hand, the accuracy of the method for intra- and inter-day were in the range 96.3 - 99.0 and

97.5 - 99.1 % for TOF - ChA and TOF - TCNQ, respectively.

Ruggedness

The analysis of tofisopam using two different analyst (operator) and different devices in different days were carried out to evaluate the ruggedness of the suggested spectrophotometric method. Results of the relative standard deviation was < 3.3 %, after repetitive measurements over three days and over an entire day, using two different instruments and two operators, This indicates that the proposed method has a high degree of precision.

Robustness

The optimum experimental factors that influence the absorbance was examined in order to evaluate the robustness of the proposed method. RSD was < 3.3 % under different conditions. The studied parameters were reagent concentration, reaction time, and stability of the resulting complex. These results indicate that the proposed method is fairly robust.

Application of the proposed method

The assay of the drug in a dosage form was first assessed for determination of tofisopam in pure solution to test the reliability of the method. Determination of tofisopam by proposed spectrophotometric method gave 96.8 - 99.0 and 96.6 - 98.6 % with RSD of 2.4 - 2.8 and 2.5 - 2.8 % for TOF-ChA and TOF-TCNQ, respectively (Table 3). These results show that the method has good accuracy and can be applied for determination of the drug in the dosage form.

Table 4 shows he results obtained for the determination of tofisopam in its dosage form using the proposed spectrophotometric method and compared with the official titrimetric method from Japanese Pharmacopeia [20]. The obtained data obtained by both methods are comparable.

Table 2: Intra- and inter-day results for the proposed method

CT Reagent	Drug taken (µg/ ml)	Found*± SD (µg/ ml)	R (%)	RSD (%)	Error (%)
Intra-day					
ChA	30.0	29.1 ± 0.87	97.0	3.0	3.0
	50.0	49.0 ± 1.37	98.0	2.8	2.0
	100.0	99.0 ± 2.67	99.0	2.7	1.0
TCNQ	40.0	39.1 ± 1.13	97.8	2.9	2.25
	80.0	79.1 ± 2.136	98.8	2.7	0.90
	120.0	119.0 ± 3.09	99.1	2.6	0.83
Inter-day					
ChA	30.0	28.9 ± 0.92	96.3	3.2	3.6
	50.0	48.8 ± 1.42	97.6	2.9	2.4
	100.0	98.8 ± 2.67	98.8	2.7	1.2
TCNQ	40.0	39.0 ±1.17	97.5	3.0	2.5
	80.0	79.0 ±2.21	98.8	2.8	1.25
	120.0	118.8 ± 2.97	99.0	2.5	1.0

*n = 5

Table 3: Validation of the proposed method for the determination of tofisopam

CT reagent	Added (µg/ ml)	Found* (µg/ ml)	R (%)	RSD (%)	Error (%)
ChA	25.0	24.2 ± 0.68	96.8	2.8	3.2
	50.0	49.0 ± 1.27	98.0	2.6	2.0
	75.0	74.0 ± 1.85	98.6	2.5	1.3
	100.0	99.1 ± 2.38	99.1	2.4	0.9
	125.0	124.5 ± 2.99	99.6	2.4	0.4
TCNQ	30.0	29. 0 ± 0.81	96.6	2.8	3.3
	50.0	49.1 ± 1.28	98.2	2.6	1.8
	80.0	79.2 ± 2.06	99.0	2.6	1.0
	100.0	98.5 ± 2.46	98.5	2.5	1.5
	150.0	148.0 ±3.70	98.6	2.5	1.3

*n=5

Table 4: Determination of tofisopam in tablet using the suggested methods and the official titrimetric method

Parameter	ChA	DCNQ	Official method *
Label claim, 50 mg/tablet	97.8± 2.6	98.1 ± 2.8	97.85± 0.31

*Non-aqueous titration

DISCUSSION

The formation of charge transfer complex due to the interaction of n-electron donner (tofisopam) with π-acceptors (ChA, and TCNQ) which produced a colored charge transfer complex with low molar absorptivity in non-polar solvent. In contrast, in polar solvents such as acetonitrile, a full electron transfer from tofisopam (D), (n electron donor), to the acceptor moiety (A) (π-acceptors, in this case ChA and TCNQ) took place with the production of strong colored radical ions with high molar absorptivity, according to Eq 1.

$$D + A \rightleftarrows \underset{\text{complex}}{(D\text{-}A)} \rightleftarrows \underset{\text{radicals ions}}{D^{\cdot+} + A^{\cdot-}} \quad \ldots\ldots (1)$$

The separation of the donor-acceptor complex (D-A) was confirmed by the high degree of ionizing power of the polar solvent, acetonitrile [21]. The apparent color complex has different absorption maxima at 520 and 842 nm for ChA and TCNQ, respectively. The color species produced with TCNQ in acetonitrile is a bluish-green colored radical anion, showing a strong absorption maxima at three different wavelengths 742, 825 and 842 nm. These bands indicate the formation of the radical anion, TCNQ •-, which perhaps is formed by the separation of an original (donor-acceptor) complex (D-A) between the drug and acceptors.

CONCLUSION

A spectrophotometric method for the assay of tofisopam in its dosage form has been developed. The method is based on using ChA and TCNQ as π-acceptors. The validated method compares well with the official titrimetric method. The developed method is simple, economical, rapid, accurate, sensitive and should be suitable for routine quality control of tofisopam in a dosage form without any interference from excipients.

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