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UV Spectrophotometric Method Used For The Analysis Of Torsimide In Biological Fluids And Pharmaceutical Formulations

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Abstract
The simple and sensitive spectrophotometric method for the determination of Torsimide reacts with 1ml of DDQ (2, 3 –dichloro -5, 6-dicylano-1, 4-benzoquinone) by charge –transfer complex method. In this method the drug Torsimide as n-electron donors with acceptor 2, 3 dichloro-5, 6- dicyano 1,4- benzoquinone (DDQ) to form Dark red color charge-transfer complexes. This reaction is instantaneous and quantitative. The drug maximum absorbance at 460 nm and Beer's law limit was obeyed at 25-175 µg/ml. The optical characteristics of the proposed method such as molar absorptivity, sandell's sensitivity, slope and intercept were 0.54084. L.mole-1 cm-, 0.0025µg.cm-2, 0.0055 and 0.0019 the correlation coefficient is 0.9999 for Torsimide respectively. The developed method was found to be simple, specific, robust, accurate and precise for the determination of Torsimide.
Keywords: *Torsimide, DDQ, chloroform, methanol, UV Spectrophotometric Method*

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I. Introduction

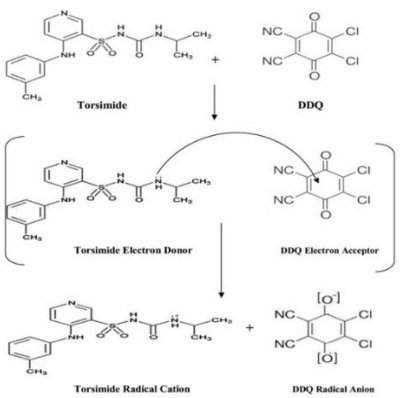
Torsimide is a loop diuretic and is chemically known as 1-{4-[3-methylphenyl) amino] pyridine-3-sulfonyl}-3-(propane -2-yl) urea [1-3]. It acts by inhibiting the Na +/k +/2Cl – carrier system in the lumen of the ascending portion of the loop of Henle, resulting in the decrease in reabsorption of sodium and chloride [4]. It is a loop diuretic mainly used in the management of edema associated with congestive heart failure and to reduce the swelling and fluid retention caused by various medical problems, including heart or liver disease [5-7].

Torsimide appears to be a useful alternative to furosimide in these patients, providing potent and long lasting diuresis while being relatively potassium and calcium sparing [8].

Dosages of torsimide of 2.5 to 5 mg/day not affect plasma rennin activity or aldosterone release to a clinically significant extent, although torsimide 20mg increases plasma rennin levels, angiotensin II activity and urinary dopamine and prostaglandin E excretion [9].

The literature survey reveals that few chromatographic methods [10-21] have been reported for the determination of torsimide in human plasma and urine. The Visible Spectrophotometric method used for the analysis of torsimide in either biological fluids or pharmaceutical formulations. The method is based on the reaction of torsimide with 2, 3- dichloro-5, 6-dicyano-1, 4-benzoquinone (DDQ) to form a red colored charged ¬transfer complex. The red colored solution is used to determine the torsimide spectrophptometrically. The reaction sequence can be shown in scheme 1.

Scheme 1



Scheme 1: The reaction sequence of charge transfer complex

Spectrum of torsimide treated with DDQ

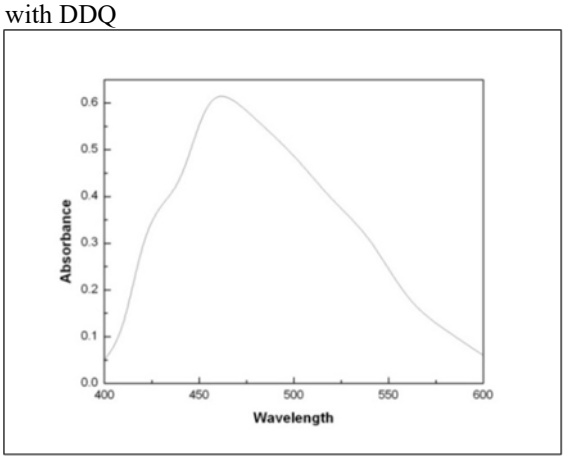


Fig 1: Spectrum of torsimide treated with DDQ solution

1.0 ml of torsimide solution (100 g/ml) and of DDQ is added to form a red colored solution. The final volume is brought to 10 ml with methanol. The resultant solution is well mixed and allowed to stand for 5 min for completion of the reaction. The absorbance of the red colored solution is measured in the wavelength range of 400 to 600 nm, against the reagent blank. The spectrum is given in fig.1.

Torsimide drug treated with DDQ solution has maximum absorbance at 460 nm fig 1. Hence, all the further studies are made at 460 nm.

The optimal conditions for the determination of torsimide are arrived at by the following steps.

Effect of concentration of DDQ solution on the absorbance of charge transfer complex is studied by the following procedure

A 1.0 ml of torsimide is taken in a series of standard flasks and varying amounts of DDQ solution are added. The contents are made up to the mark with methanol. Reaction mixture was shaken gently for 5 minutes and allowed to stand for 5 minutes to complete the reaction. The absorbance of the resultant solutions is measured at 460 nm and the data are presented in table 1.

Table 1

Volume of DDQ solution (ml)	Absorbance at 460 nm
0.5	0.458
1.0	0.576
1.5	0.580
2.0	0.720
2.5	0.572

The data in table 1 indicate that 2.0 ml of DDQ is necessary for achieving maximum absorbance and hence maintained throughout the experimental studies

Assay Procedures

Various aliquots of the standard torsimide solution ranging from 0.2-1.6 ml are transferred into a series of standard flasks. To each flask, 2.0 ml of DDQ solution is added to produce a red color. The final volume is brought to 10 ml with methanol. The reaction mixture in each flask is well shaken and allowed to stand for 5 minutes to complete the reaction. The absorbance of the red colored solution is measured at 460 nm, against the reagent blank prepared in similar manner omitting drug solution. Calibration Graph is obtained by plotting absorbance values against the concentration of torsimide solution. The calibration curve is found to be linear over a concentration range of 25 to 170 g/ml of torsimide the amount of torsimide present in the sample is read from the calibration graph. The results are presented in fig 2.

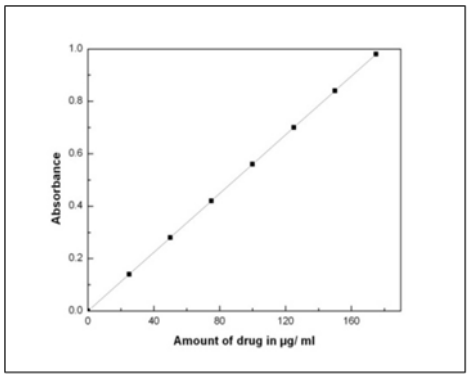


Fig 2: Calibration Curve of torsimide

Assay of torsimide in pharmaceutical formulation

The determination of the drug from the marketed tablet formulations was carried out by the following procedure. Tablets are weighed and contents are powdered and well mixed. Thepowder equivalent to 50 mg of torsimide is dissolved in methanol, filtered, residue is washed with distilled water and the volume is made up to 50 ml with methanol. Further, dilution is made as described in the preparation of standard solution of torsimide. Further the analysis is carried out as per procedure described above and results are summarized in the table 4. The amount of drug present in the sample is estimated from calibration graph.

II. Method Validation

1. Linearity

For the quantitative analysis of the drug torsimide, the method was validated according to ICH guidelines and the following characters of validation are addressed Linearity, Accuracy, Precision, Specificity, LOD, LOQ and Robustness. The experimental conditions of the drug torsimide by spectrophotometric method, standard calibration curves with DDQ were constructed by plotting absorbance versus concentration. The statistical parameters were given in the regression equation calculated from calibration plots along with standard deviation. The linearity of calibration graphs are proved by high values of correlation coefficient and small values of y-intercept of the regression equation. The molar absorbtivities of the colored complexes and relative standard deviation for the proposed spectrophotometric method were also calculated and shown in table 2.

2. Robustness and Ruggedness

In the study of robustness, some parameters like PH range, concentrations of the drug reagents and shaking time were interchanged. Even after that, the results were unaffected by small deliberate and shaking time. The method of Ruggedness was expressed as the percentage of relative standard deviation for the proposed method developed by two analysts in two different instruments in two different days. The results proved that there is no statistical difference between the above said two analysts and instruments which conclude the developed analytical method were robust and rugged.

3. Accuracy

Recovery studies were carried out to study the accuracy of the proposed analytical method in bulk drug and in biological fluids, viz. Serum and urine which were given in Table 2. All the results are good within the acceptable boundary. The percentage recovery was calculated as,

Percentage Recovery = [(a-b)/c] x 100.

Where ‘a’ is the total amount of the drug estimated.

‘b’ is the amount of the drug found on pre-analyzed basis (standard drug solution).

‘c’ is the amount of the pure drug added to the formulation.

4. Precision

The intra-day and inter-day precision was determined by analyzing the same concentration of the solutions on three different days. The precision calculated as inter-day and intra-day RSD % is less than 1 proves that there is no considerable difference for the assay which is tested in inter-day and intra-day from pharmaceutical ingredients and biological samples. The results are presented in table 3.

5. Limit of detection (LOD) and Limit of Quantification (LOQ)

LOD and LOQ were determined by using the formula based on the standard deviation of the response and slope. LOD and LOQ were calculated by using the equations. LOD= 3x s/S and LOQ = 10xs/S.

where‘s’ is the standard deviation of the intercept and S is the slope.

6. Recovery

According to the proposed analytical method, the drug samples of different concentrations were analyzed spectrophotometrically and the plots were found to be linear, accurate, precise and selective by running three replicates of each concentration measured for three days. The average recoveries were recorded in table 3.

7. Effect of interferences

The effect of the recipients, viz. Glucose, Sucrose, Lactose, Dextrose, Talc and Starch which frequently come with the drug torsimide in its dosage forms was studied to prove the importance of the proposed analytical method. The results showed that there is no interference from the degradation which indicates a high selectivity in determining torsimide in its dosage form. These results are recorded in table 5.

8. Assay in serum and urine samples

The collected blood and urine samples from donors were centrifuged at 3000 rpm for nearly 10 min. The resulted solutions were filtered and preserved in the absence of light at a temperature of 4oC. From these solutions, various concentrations of the drug torsimide were analyzed with the help of proposed analytical method and these results were recorded in table 56. Hence, the proposed method can be successfully applied to recover torsimide in biological samples, viz. urine and serum due to its high accuracy and good recoveries.

9. Results and discussion

The optimum conditions were established by changing one parameter at a time and keeping the others constant and by observing the effect produced on the absorbance of the colored species.

In this method, the drug reacts with DDQ solution to form an orange red charge complex. The red colored charge complex solution formed is measured at 460 nm against reagent blank. The amount of drug read from calibration curve. The calibration curve is linear over the range of 25-175 □g/ml of torsimide. The optical characteristics of the proposed method such as absorption maxima, Beer’s law limits, molar absorptivity and Sandell’s sensitivity values show that the method is sensitivity. The regression analysis using method of least squares was made for the slope (b), intercept (a) and correlation (r) obtained from different concentrations and results arew summarized in the table 2 The value of correlation coefficient (r) was 0.999, which indicated the good linearity of calibration lines. The percent relative standard deviation calculated from the five measurements of torsimide shown in table 4. The % RSD is less than 2, and indicates that the method has good reproducibility. The values of standard deviation values are low indicates high accuracy and reproducibility of the method. The‘t’ calculated values are compares well with the theoretical value of 2.78 there by indicating that the precision of the method. There is no effect of additives and recipients such starch, calcium lactose and glucose in the concentrations those present in general pharmaceutical preparations.

The proposed method is found to be simple, precise, accurate, time saving, reproducible and can be conveniently adopted for routine analysis of estimation of torsimide in bulk drugs samples and pharmaceutical formulations.

Table 2

Parameters	Proposed method
λ max(nm)	460
Beer’s law limit(µg/ml)	25-175
Molar absorptivity (L.mole^{-1} cm^{-1})	0.54084
Sandal’s sensitivity (µg.cm^{-2}/0.001 A.U)	0.0025
Slope(b)	0.0055
Intercept(a)	0.0019
Correlation coefficient(r)	0.9999
Relative standard deviation(RSD)%	0.1785
LOD(µg/ml)	0.5412
LOQ(µg/ml)	1.8023
Color	Dark red

Evaluation of interday and intraday accuracy

Table 4

Tablets	Labelled amount mg/ml	Amount found mg/ml	%Recovery	±SD	% RSD
Torsemi	5	4.96	99.33	0.0577	0.1863
Torsinex	10	9.93	99.33	0.288	0.2906
Torsed	15	14.94	99.64	0.0450	0.3016

*Average of five determination based on label claim

Table 5

Excipients	Amount taken mg/ml	*Found mg/ml	Recovery %	±SD	RSD %
Glucose	5	4.98	99.60	0.01	0.2008
Sucrose	10	9.99	99.80	0.01	0.1002
Lactose	15	14.97	99.73	0.01	0.0668
Dextrose	20	19.46	99.23	0.056	0.8509
Talc	20	19.97	99.25	0.56	0.8648
Starch	30	29.95	99.87	0.01	0.0509

* Average of five determinations

Table 6

Sample	Added mg/ml	*Found mg/ml	Recovery %	±SD	RSD %
Serum samples	0.4	0.39	99.58	0.0040	0.2482
	0.6	0.59	99.55	0.0020	0.3484
	0.8	0.79	99.58	0.0020	0.2612
	2.0	1.98	99.41	0.0076	0.3841
Urine samples	0.5	0.49	99.06	0.001	0.2317
	0.7	0.69	99.71	0.001	0.1432
	0.9	0.89	99.06	0.002	0.2229
	1.1	1.09	99.75	0.002	0.1897

*Average of five determinations

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