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RESEARCH ARTICLE

Spectrophotometric Determination of Drugs in Bulk and Pharmaceutical Dosage Forms by Forming CT Complexes with DDQ

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ABSTRACT

A simple and sensitive UV-Visible spectrophotometric method is described for the estimation of four drugs viz., Brinzolamide, Olanzapine, Donepezil and Vilazodone in their pure form as well as in some method of pharmaceutical dosage forms using Dichloro-5,6-dicyano-1,4-benzoquinone (DDQ) as analytical reagent. The formation of charge transfer complexes of drugs as n-electron donor with DDQ as π-acceptor is the basis for determination of drugs. Acetonitrile was found to be suitable solvent for the analysis. The methods have been validated in terms of ICH guidelines. Under the optimized experimental conditions, Beer's law is obeyed over the concentration ranges of 14-84 µg/ml, 2-12 µg/ml, 90-540 µg/ml and 20-120 µg/ml for Brinzolamide, Olanzapine, Donepezil and Vilazodone respectively. The proposed methods for applied to the determination of the cited drug both in bulk and pharmaceutical preparation.

KEYWORDS

Spectrophotometry, DDQ, Drugs, Quantification, Validation

INTRODUCTION

Brinzolamide

Brinzolamide, [(R)-(+)-4-Ethylamino-2-(3-methoxypropyl)-3, 4-dihydro-2H Thieno [3,2-e]-1,2-thiazine-6-sulfonamide-1,1-dioxide], (Figure 1) is a new active substance which is useful only for topical use in the treatment of glaucoma. It is used to lower intra-ocular pressure in patients with open-angle glaucoma or ocular hypertension. There are UV[1] and HPLC[2] method available for simultaneous estimation of Brinzolamide and timolol. Several analytical methods based on UV, RP-HPLC, HPTLC, LC-QTOF-MS/MS[3] methods were reported for the determination of Brinzolamide alone.

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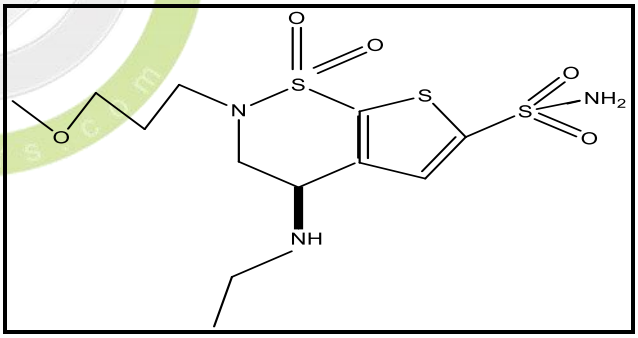

Figure 1: Chemical structure of Brinzolamide

Olanzapine (OLP)

Olanzapine chemically known as 2-methyl-4- (4-methyl-1-piperazinyl)-10H-thieno [2, 3-b][1,5] benzodiazepine (Figure 2), is an atypical antipsychotic agent, also known as second-generation antipsychotic (SGA)[4]. Since its introduction in 1996 in over 84 countries, several workers have reported HPLC methods for the determination of OLP in plasma, serum, human

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breast milk and rat brain[5-13]. A survey of literature showed HPTLC[14], UV[15] and Linear voltammetry[16] have reported for the assay of OLP in pharmaceuticals.

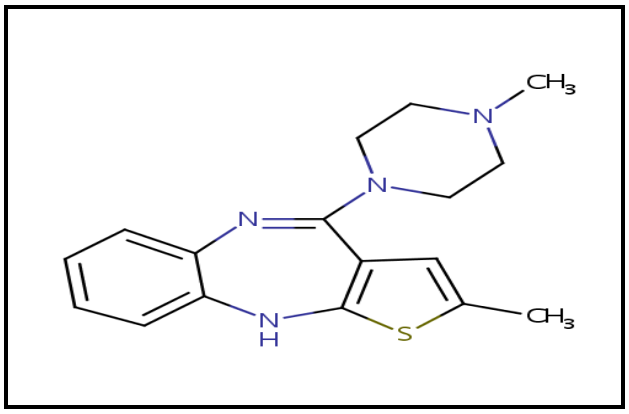

Figure 2: Chemical structure of Olanzapine

Donepezil

Donepezil hydrochloride, chemically 2, 3-dihydro-5, 6- dimethoxy-2-[[1-(phenylmethyl)-4-piperidinyl] methyl]- 1-H-inden-1-one hydrochloride (Figure 3) is clinically used worldwide for patients with mild to severe Alzheimer disease[17]. The most commonly used techniques for the determination of Donepezil hydrochloride are LC[18] and RP-HPLC[19] methods.

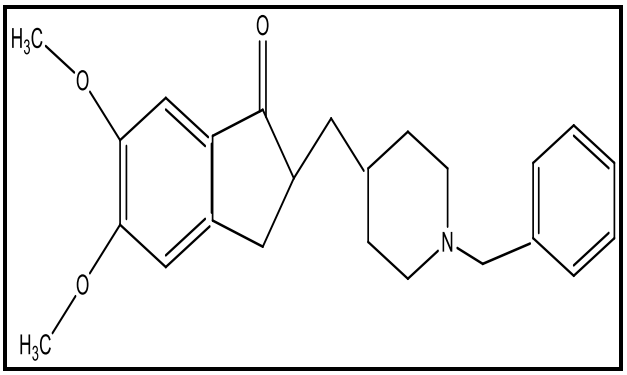

Figure 3: Chemical structure Donepezil

Vilazodone

Vilazodone IUPAC Name is 5-(4-[4-(5-cyano-1H-indol-3-yl)butyl]piperazin-1-yl) benzofuran-2carbox amide. It belongs to the category serotonergic antidepressant. Vilazodone was approved by the FDA for use in the United States to treat major depressive disorder in January 21, 2011. Vilazodone acts as a serotonin reuptake inhibitor and 5-HT1A receptor partial agonist. It has negligible affinity for other serotonin receptors such as 5-HT1D, 5-HT2A, and 5-HT2C[20]. There are very few methods reported for the determination of Vilazodone viz. UV[21] method, spectrofluorimetric and also an RP-HPLC[22] methods were reported for its estimation in bulk and pharmaceutical formulation.

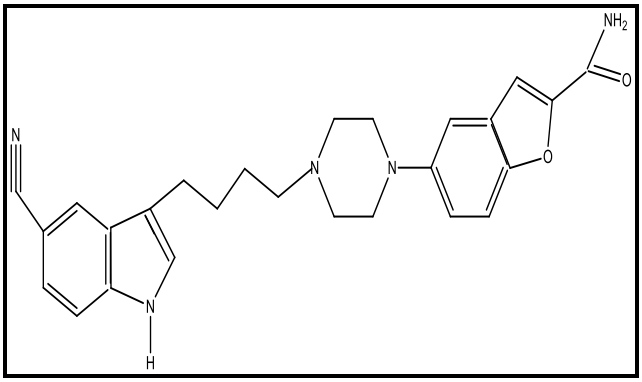

Figure 4: Chemical structure Vilazodone

Charge transfer phenomena were introduced by Mulliken and widely discussed by Foster to define a new type of adducts. Molecular interactions between electron donors and acceptors are generally the formation of intensely colored charge transfer complexes which have absorbance in visible region. Charge transfer interactions within the formation of molecular complex involving a resonance with a transfer of charge from an electron donor (D) to an electron acceptor (A) were also showed by Mulliken.

$$D+A \leftrightarrow D^{\cdot+}+A^{\cdot-}$$

Many drugs are easy to determine by spectrophotometry based on colour charge transfer (CT) complexes formed between electron acceptors, either π- or δ-acceptors and drugs as electron donors either n or π donors.

The present investigation aims to develop more sensitive, simple, eco friendliness and cost effective methods for the determination of these drugs in pure form and in dosage form. Using DDQ as π acceptor based on the formation of charge transfer complexes.

MATERIALS AND METHODS

Instrumentation

Shimadzu 2600 double beam UV-Visible spectrophotometer is used to record the spectra of

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individual components as well as the charge transfer complexes using matched pair of Quartz cells of 10mm path length.

Materials

The 2,3-Dichloro 5,6-Dicyanobenzoquinone is supplied by sigma Aldrich. The AR grade solvent acetonitrile is supplied by SD fine chem. Ltd. Mumbai, India. These drugs were supplied by MSN Laboratories Ltd.

Standard and Sample Preparation

An accurate weight of drugs (100 mg) were dissolved in 100 ml of acetonitrile to give concentrations of 1000 µg/ml and further diluted according to the requirement for their analysis.

Method

Four drugs are found to respond to DDQ in acetonitrile viz., Brinzolamide, Olanzapine, Donepezil and Vilazodone. Into a series of 10ml volumetric flask 0.5 to 3ml aliquots of drugs were transferred. To each flask 1ml of ($4.4X10^{-3}$M) DDQ solution in acetonitrile was added and remaining volume was made up by solvent. The absorbance of the solution was measured after 15min of mixing at 585 nm.

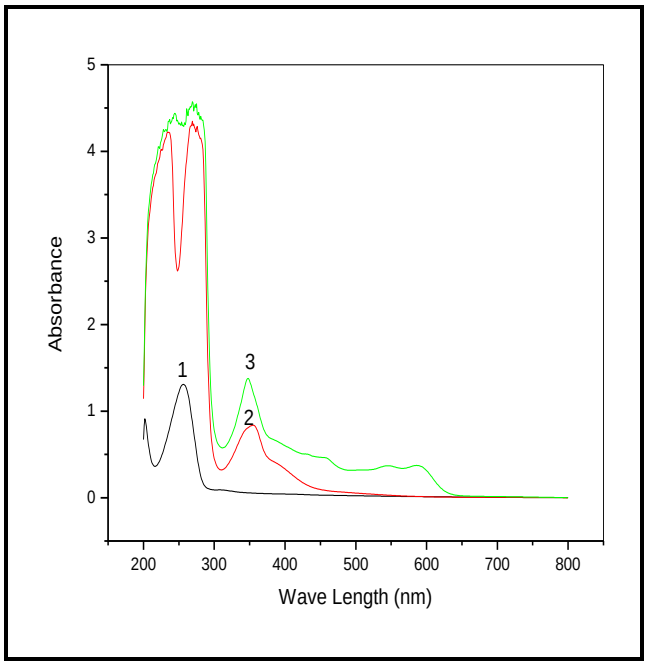

Figure 5: (1) Brinzolamide in Acetonitrile, (2) DDQ in Acetronitrile (3) Charge transfer complex of Brinzolamide with DDQ

RESULTS AND DISCUSSION

The DDQ solution of 1 mg/ml ($4.4X10^{-3}$M) in acetonitrile was freshly prepared. Aliquots of drugs (0.5 – 3.0) were transferred into a series of 10ml calibrated flasks, to each flask, 1ml of DDQ solution in acetonitrile was added and remaining volume was made up by solvent. The absorbance of thick brown colored solution was recorded after 15min of mixing against reagent blank at 585nm is plotted absorbance versus the corresponding concentrations (µg/ml) of the drug to construct the calibration curve Figure 6. Calibration curves were linear for all the drugs whose limits are mentioned in table (1). Slope intercept, correlation coefficient of the calibration curves are calculated and tabulated.

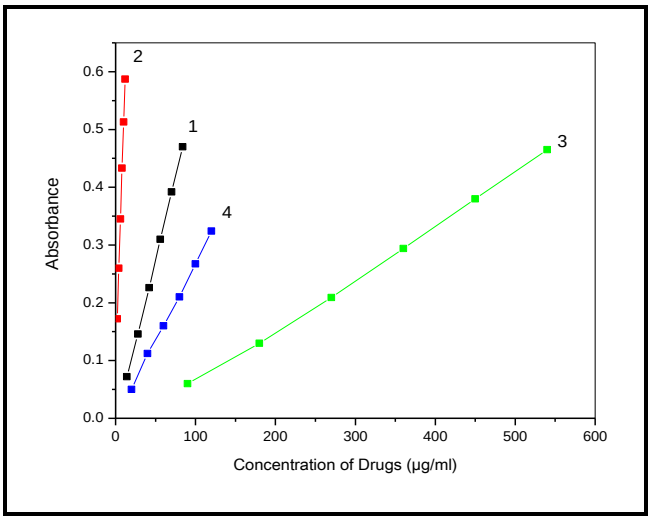

Figure 6: Calibration curves of DDQ with (1) Brinzolamide, (2) Olanzapine, (3) Donepezil and (4) Vilazodone

Determination of Drugs in Dosage form

Ten tablets were weighed, finely powdered and an accurately weighed quantity o f the powdered tablet contents equivalent of 50mg of the active ingredient was transferred into a 50 ml calibrated flask and dissolved in about 50ml of methanol. The contents of the flask were swirled, sonicated for 10 minutes. The mixture was filtered and evaporated to dryness. Residue was dissolved in acetonitrile heating of water bath for the complete dissolution of drug.

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Table 1: Spectral, analytical and statistical parameters of charge transfer complexes of drugs with DDQ

Drugs Name Parameters	**Brinzolamide**	**Olanzapine**	**Donepezil**	**Vilazodone**
λ max, nm	585	585	585	585
Beer's law limit (µg/ml)	14-84	2-12	90-540	20-120
Molar absorptivity (L mol^{-1} cm^{-1})	1998.36	13434	946.86	1235.90
Slope(specific absorptivity), b	0.0057	0.0418	0.01	0.0027
Intercept, a	0.0118	0.0928	0.0297	0.0012
Correlation coefficient, r	0.9997	0.9991	0.9987	0.9988
Sandal's sensitivity (µg cm^{-2})	0.030	0.2	0.0326	0.5
Formation constant , K(M-1)	811	4917	263	164
Standard deviation of intercepts (n=6)	0.0128	0.08	0.019	0.0020
Limit of detection (µg/ml)	7.41	6.315	6.27	2.4
Limit of quantification (µg/ml)	22.45	19.51	19	7.407
Regression equation Y=a+bx ; x=conc. of drug((µg/ml)	0.0118 + 0.0057x	0.0928 + 0.041x	0.0297 + 0.01x	0.0012 + 0.0027x

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The solution obtained was diluted with acetonitrile to obtain a concentration in the range of linearity previously determined with pure drug. This is common for all drugs.

Effect of Concentration of Acceptor

To establish the optimum concentration of reagent Brinzolamide 14µg/ml, Olanzapine 2-12 µg/ml, Donepezil 90 µg/ml, Vilazodone 20 µg/ml were react with different volumes of DDQ ($4.4X10^{-3}$M). The results showed that the highest absorbance was obtained with 1ml. Hence 1ml of reagent was used for the determination of drugs (Figure 7).

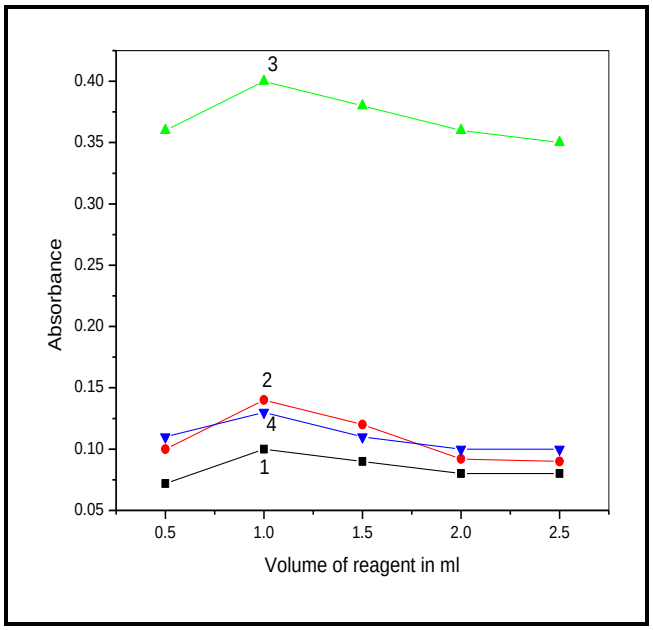

Figure 7: Effect of volume of reagent on the optical density of the charge transfer complex of DDQ and (1) Brinzolamide, (2) Olanzapine, (3) Donepezil and (4) Vilazodone

Effect of Solvent

Both polar and non-polar solvents such as methanol, acetone, chloroform, 1, 2-dichloroethane and acetonitrile were used to select elegant solvent for the analysis of drug.

Acetonitrile is found to be suitable solvent for DDQ, which produces maximum absorbance with a fixed concentration of drugs, while other solvents produced lower absorbance due to incomplete dissociation of complex. Hence acetonitrile is used throughout the study.

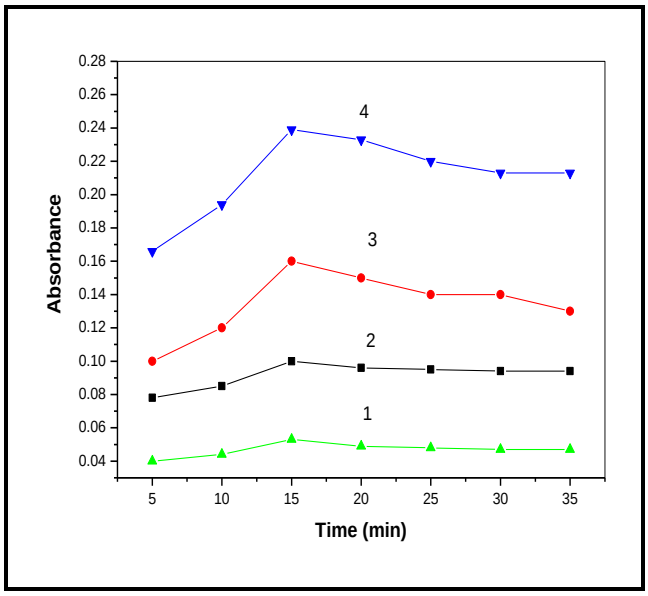

Figure 8: Effect of reaction time on formation of charge transfer complexes of DDQ and (1) Brinzolamide, (2) Olanzapine, (3) Donepezil and (4) Vilazodone

Effect of Reaction Time

The interaction of DDQ with drugs resulted in the formation of colored product with stabilized within 15 minutes of mixing. The developed color remained stable at room temperature for about an hour. After a day all solutions decolorized (Figure 8).

Method Validation

The methods developed have been validated in terms of guidelines of international conference of harmonization (ICH) viz., sensitivity, selectivity, precision, accuracy, linearity, LOD, LOQ sandell's sensitivity and robustness. The precision is tested by repeating each experiment at least 6 times while the accuracy has been tested by taking known weight of sample and performing recovery experiments. The values %RSD and t- and F tests are in the permissible range of experimental errors. (Table2).

$$LOD = 3.3\ s/S$$

$$LOQ = 10\ s/S$$

Where s = standard deviation of the intercept (n=6)

S = slope of Calibration plot

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Table 2: Application of proposed method for the analysis of drugs in their pure form

Drugs Name Parameters	**Brinzolamide**	**Olanzapine**	**Donepezil**	**Vilazodone**
Amount taken (µg/ml)	14 28 42 56	2 4 6 8	90 180 220 310	20 40 60 80
Amount found (µg/ml)	13.97 27.98 42.01 55.99	1.95 3.96 6.02 7.98	89.99 180.02 220.01 309.04	20.01 39.97 59.9 79.92
% Recovery	99.45 99.98 100.12 99.94	99.89 99.9 100.14 99.85	99.97 100.03 100.05 99.6	100.12 99.97 99.98 99.9
% RSD	0.076 0.41 0.019 0.312	0.09 0.4 0.55 0.12	0.131 0.08 0.049 0.1	0.4 0.01 0.15 0.7
Proposed mean ± SD	99.87±0.076	99.86±0.09	99.91±0.131	99.99±0.04
Ref Mean ± SD	99.09±1.000 (n=5)	102.3± 1.76 (n=3)	100.35±0.76 (n=6)	100.01±0.79 (n=6)
t-test	2.061 (1.943)	1.606 (1.134)	1.997 (2.447)	2.322 (2.447)
F-test	0.0057 (3.107)	0.0026 (3.288)	0.0297 (3.054)	0.00256 (3.054)

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Table 3: Application of proposed method for the analysis of studied drugs in their pharmaceutical form

Drugs Name Parameters	**Brinzolamide**	**Olanzapine**	**Donepezil**	**Vilazodone**
Amount taken (µg/ml)	28 42 56 70	4 6 8 10	180 270 360 450	40 60 80 100
Amount found (µg/ml)	27.99 42.02 56.07 70.97	3.97 5.98 8.03 9.99	180.01 269.99 359.97 450.99	40.02 59.97 79.95 99.98
% Recovery	99.98 100.05 100.47 99.93	99.6 99.85 100.13 99.81	100.023 99.89 99.95 99.91	100.01 99.95 99.96 99.98
% RSD	0.620 0.415 0.09 0.5	0.35 0.4 0.39 0.31	0.532 0.6 0.71 0.39	0.128 0.1 0.8 0.15
Proposed mean ± SD (n=6)	100.10 ± 0.620	99.84 ± 0.35	99.94 ± 0.532	99.97 ± 0.128
Ref Mean ± SD	100.2 ± 1.37 (n=3)	104.1 ± 1.26 (n=5)	100.83 ± 0.38 (n=6)	99.64 ± 0.82 (n=3)
t-test	0.903 (1.134)	1.925 (1.943)	0.570 (2.447)	0.652 (1.134)
F-test	0.204 (3.288)	0.077 (3.107)	1.96 (3.054)	2.43 (3.282)

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The robustness of the methods was examined by performing the experiments on 3 different spectrophotometers with excellent tally of absorbance values.

The method developed has also been applied for the analysis of pharmaceuticals. The recovery experiments performed show high accuracy and precision and the results are compared with the available validated reported methods on these drugs. The values %RSD and t-and F tests are in the permissible range of experimental errors (Table3). And show that the methods can be used in both pharmaceutical and drug industries.

Stability Constants of Charge Transfer Complexes

Benesi – Hildebrand method (BH) is used for determination of stability constant K and molar absorption coefficient of the charge transfer complexes.

Ao/d = 1/ K (Do) € +1/ €

Where Ao = conc. Of acceptor, d= optical density, Do = conc. Of drug, €= Molar absorption coefficient and K= stability constant.

A plot of Ao/d Vs 1/Do is a straight line from whose slope and intercept the K and € are determined.

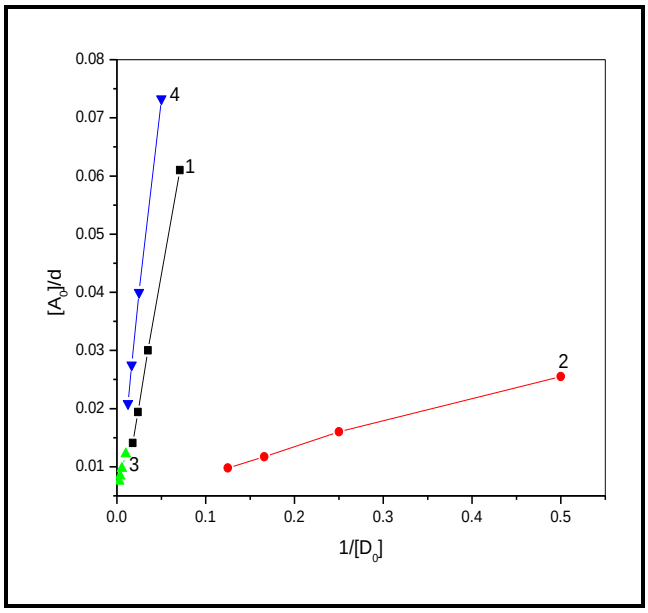

Figure 9: Benesi-Hildebrand plot of DDQ with (1) Brinzolamide, (2) Olanzapine, (3) Donepezil and (4) Vilazodone

Stoichiometry

The stoichiometry of each of the complex has been determined from Job's continuous variation method and found to be 1:1 in each case. A typical Job's plot of selected drugs with DDQ is presented in (Figure 10).

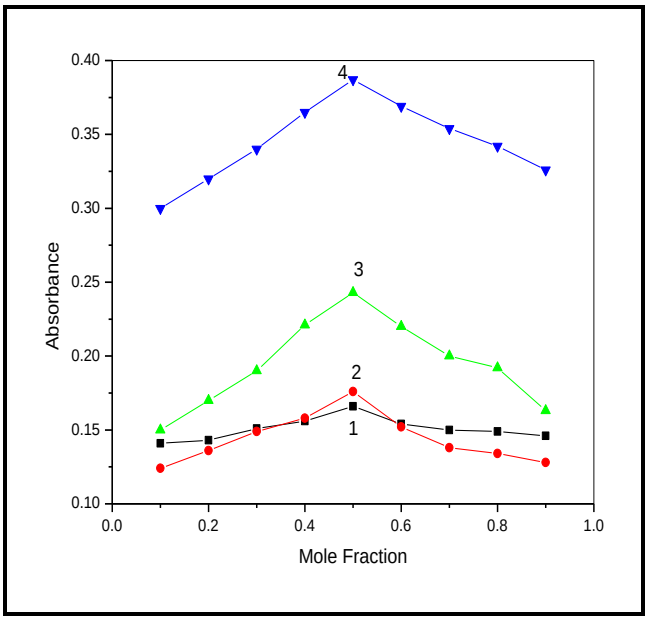

Figure 10: Continuous variation plot of DDQ with (1) Brinzolamide, (2) Olanzapine, (3) Donepezil and (4) Vilazodone

CONCLUSION

DDQ forms charge transfer complexes with the above selected drugs and exhibits at 585nm. By using this CT complex method the obtained results are simple, accurate, precise, ecofriendly and robust for estimation of these drugs. Proposed methods are successfully applied for routine analysis of pure drug and pharmaceutical dosage forms.

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