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Spectrophotometric Determination of Drugs Using 2,3-Dichloro 5,6-dicyano *p*- benzoquinone as Analytical Reagent

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ABSTRACT

Six drugs *viz.,* Astemizole, Domperidone, Esomeprazole, Losartan potassium, Sumatriptan and **Terazosin** were tested for the formation of charge transfer complexes with 2, 3-dichloro 5, 6-dicyano- *p*- benzoquinone, (DDQ). Each of these drugs turned the pale yellow colour of reagent *i.e* DDQ. in CH_3CN, to purple and exhibited three bands at 455, 545 and 588 nm due to anion of the reagent whose intensity increased with increase in the concentration of the drugs and formed a basis for quantitative determination of the drugs. The complexes were found to have 1:1 composition and have stability of the order 10^3 to 10^4 The effect of the concentration reagent, polarity of solvent, interference of excipients have been studied and optimized. Acetonitrile was found to be suitable solvent for the analysis. The methods have been validated in terms of ICH guidelines and applied to the quantification of pharmaceuticals. The variation of slopes of calibration plots and stability constants of the complexes are discussed in terms of structures of the drugs.

Key words: Spectrophotometry, DDQ, Drugs, Quantification, Validation.

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INTRODUCTION

2,3-Dichloro 5,6- dicyano- *p*-benzoquinone(DDQ) is an oxidizing [1], dehydrating agent [2] in synthetic organic chemistry as well as it is known for its interaction with drugs having donor sites in their structures, and form ion-pair charge transfer complexes which offers a basis for quantification of the drugs [3-6].

Thorough survey of literature on the following drugs revealed that quantification using DDQ as analytical reagent has not been reported yet although the reagent is common, known to offer simple, sensitive method of quantification for drugs. This prompted the authors to develop quantification methods for the following drugs, (Scheme 1), using DDQ as a chromogen and hence tested them for the formation of charge transfer complexes which is expected to form a basis for the quantification of the drugs The physiological activity of the drugs and methods used so far for their quantification are:

Astemizole, chemically 1 - [(4 – fluorophenyl) methyl] – N - [1 - [2 - (4 - methoxyphenyl) ethyl] -4 piperidyl] benzoimidazol -2- amine (Scheme.1a) is a long-acting, selective histamine H1 receptor antagonist. It is a second generation antihistamine in that it does not readily cross the blood-brain barrier. Astemizole is used in the treatment of both seasonal and perennial allergic rhenitis, allergic conjunctivitis, chronic urticaria and other chronic allergies. Because of its physiological significance, it has been quantified by several methods which are enumerated in the recent reference [7].

Domperidone, chemically is [5-chloro-1-{1-[3-(2-oxobenzimidazolin-1-yl)-propyl]-4-piperidyl} benzimidazoline-2-one] (Scheme.1b). It is a synthetic benzimidazole compound that acts as a dopamine D2 receptor antagonist. Its localization outside the blood –brain barrier and antiemetic properties has made it is a useful adjunct in therapy for Parkinson's disease. There has been rehabilitated curiosity in antidopaminergic prokinetic agents, a 5-HT4 agonist, from the market. Domperidone is also as a prokinetic negotiator for treatment of upper gastrointestinal motility disorders. It continues to be an attractive alternative to metoclopramide because it has fewer neurological side effects. Patients receiving Domperidone or other prokinetic agents for diabetic gastropathy or gastroparesis should also be managing diet, lifestyle, and other medications to optimize gastric motility.]. Several methods have been reported for determination of Domperidone all of which are reviewed in s recent reference [8].

Esomeprazole magnesium, chemically known as 6-methoxy-2-[(4-methoxy-3,5-dimethylpyridin-2-yl)methylsulfinyl]-1H-benzimidazole, (Scheme.1c) is the first proton pump inhibitor developed as a single optical isomer of Omeprazole used for the treatment of acid releases base that is concentrated in the acidic compartment of secretory canaliculus of the parietal cell where it undergoes acid-catalysed transformation to diseases. The drug is used in the management of patients with gastroesophageal reflux disease, erosive reflux esophagitis and peptic ulcer. The drug is a weak tetracyclic achiral cationic sulphenamide. This then reacts with specific cysteines resulting in the inhibition of the H^+/ K+ - APTase enzyme. Esomeprazole

magnesium has been studied and determined by several procedures and are exhaustively reviewed [9].

Losartan potassium (Scheme.1d) chemically 6-methoxy-2-[(4-methoxy-3,5-dimethylpyridin-2-yl)methylsulfinyl]-1H-benzimidazole is widely used for the treatment of hypertension and cardiovascular diseases in combined pharmaceutical preparations. Losartan potassium and its principal active metabolites block the vasoconstrictor and aldosterone-secreting effects of angiotensin II by selectively blocking the binding of angiotensin II to angiotensin II receptor type 1 (AT1) receptor found in many tissues (vascular smooth muscle, adrenal gland). Losartan potassium has been studied and determined by several procedures and are exhaustively reviewed [10].

Sumatriptan with chemical name as 1-[3-(2-dimethylaminoethyl)-1H-indol-5-yl]-N-methyl-methanesulfonamide (Scheme.1e) is 5-HT1 receptor agonist used in the treatment of migraine. The side effects are severe nausea or vomiting during their migraine attack. Methods of determination of sumatriptan used so far are presented in a recent reference [11].

Terazosin (Scheme.1f), chemically known as alpha-1-selective adrenoceptor blocking agent, is a quinazoline derivative which is used to treat hypertension (high blood pressure) and benign prostatic hyperplasia (enlarged prostate). It causes the blood vessels (veins and arteries) to relax and expand, improving blood flow. Terazosin also relaxes muscles in the prostate and bladder neck, making it easier to urinate. Previously reported methods of analysis of the amount of a medicine in its pharmaceutical formulation have been cited in the reference [12].

EXPERIMENTAL

INSTRUMENT

The spectra of individual components and charge transfer complexes were recorded on Shimadzu 140 double beam spectrophotometer as well as on Thermo Nicolet 100 & Elico 159 UV- Visible single beam spectrophotometers using matched pair of Quartz cells of 10mm path length.

MATERIALS

DDQ was obtained from Sd Fine Chem India Ltd.(mp 213-214^0 C). It was recrystallised twice from a 3:1 mixture of chloroform and benzene. A stock solution of 60mg/100ml w/v (2.6 x 10^{-3} *M*) in acetoniltrile was freshly prepared. . The drugs used in study are procured from hetero drugs pvt.ltd. Hyderabad. Most of the drugs procured are in the form of their acid salts. They have been neutralized by adding calculated amount of NaOH/NH_4OH as required followed by extraction with ether or $CHCl_3$. They were recrystallized from suitable solvent till TLC pure... Stock solutions of drugs are prepared first (1mg/ml) and are further diluted according to the requirement for their analysis.

The materials used are spectrograde acetonitrile, AR grade methanol, ether, NaOH and NH_4OH all of them are supplied by S D fine chemicals, Mumbai.

Extraction of drugs for pharmaceutical analysis.

Astemizole

Twenty tablets (Stemiz – 10mg) were finely powdered and mixed. An accurately weighed 50mg of the drug was transferred into a volumetric flask and 50ml of chloroform was added and shaken well for 5 minutes. The content was filtered using a quantitative filter paper in a beaker. The residue was washed with 20ml of chloroform. Chloroform was evaporated by heating on water bath. To the content 1, 2-dichloroethane or acetonitrile was added and serial dilutions are done accordingly.

Domperidone

Ten capsules (Domcolic – 10mg) were weighed accurately. The average weight was determined and then ground to a fine powder. A quantity equivalent to 50 mg Domperidone was transferred into a 100 ml volumetric flask. The contents were ultrasonicated for 10 min with 50 ml of methanol. Then the solution was filtered through a Whatman filter paper (No. 40). The residue was washed with methanol. The methanol is evaporated and to the content acetonitrile or 1, 2-dichlorethane is added and heated on water bath for some time for complete dissolution of the drug. Then serial dilutions are done up to the requirements.

Esomeprazole

Twenty capsules (Esofag – 20mg) were weighed accurately. The average weight was determined and then ground to a fine powder. A quantity equivalent to 50 mg Esomeprazole was transferred to a 100 ml volumetric flask. The contents were ultrasonicated for 10 min with 50 ml of methanol, made to volume with methanol. Then the solution was filtered through a Whatman filter paper (No. 40). The residue was washed with methanol. The methanol is evaporated and to the content acetonitrile is added and heated on water bath for some time for the complete dissolution of the drug. Then serial dilutions are done up to the requirements.

Losartan K

Twenty tablets (Lartan – 25mg) were weighed accurately and powdered. An amount of the powder equivalent to 500 mg of losartan was dissolved in 50 ml of methanol. The solution was ultrasonicated for 10 minutes. Then, the solution was filtered through Whatman filter paper No. 41. The filtrate was evperated and residue was dissolved in acetonitrile. Aliquots of these solutions were used in such a way that the concentration of each drug was within the range of the calibration matrix. The diluted solutions were analyzed six times.

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Sumatriptan succinate

Batches of ten tablets (Sumitrex-25mg) were crushed in a glass mortar after which an amount equivalent to 50 mg of the salt was accurately weighed out. The amount weighed out was then dissolved in sufficient volume of water and filtered with Whatmann filter paper. The residue was washed with a few milliliters of water. The filtrate is taken in separating funnel containing ether and 0.1 N NaOH solution is added for neutralization. The content is shaken for 5 minutes. Organic layer gets separated and extraction continued in two portions with ether. Ether is evaporated to dryness and acetonitrile is added to prepare stock solution and diluted accordingly.

Terazosin

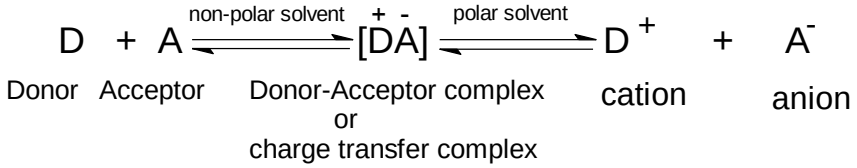
Ten tablets (Olyster – 5mg) were crushed into powder. An equivalent of 50mg was dissolved in double distilled water. Then, the aqueous solution was filtered to separate insoluble solids. To the clear solution obtained, dilute ammonia solution was added. The drug was separated as solid. The solid was extracted with ether and evaporated on waterbath. The residue obtained was dissolved in acetonintrile which was serially diluted for analysis.

SPECTRA

The spectra of ion – pair Charge transfer complexes were recorded in CH_3CN for quantification studies as well as to evaluate other parameters like stability constants and stoichiometry of the complexes from absorption studies on characteristic absorption band of anion of the acceptor. The spectra of each sample at 2 or 3 different concentration have been recorded on scan mode and for the remaining optical density were noted on fixed mode.

RESULTS AND DISCUSSION

DDQ is a strong π- electron acceptor having electron affinity 1.9 eV which interacts instantaneously with the basic nitrogenous compounds to form charge transfer complexes of n – π type [13]. DDQ solution in acetonitrile displayed a maximum absorption peak at 350nm while all the drugs analysed in the present study showed a maximum absorption peak below 250 nm. Mixing the solutions of drug and the solution of DDQ in acetonitrile yields intense reddish brown color and causes an immediate change in the absorption spectrum with new peaks at 455, 545 and 588 nm (Fig. 1). The spectral and analytical parameters of ion pair complexes of DDQ with drugsare presented in Table 1



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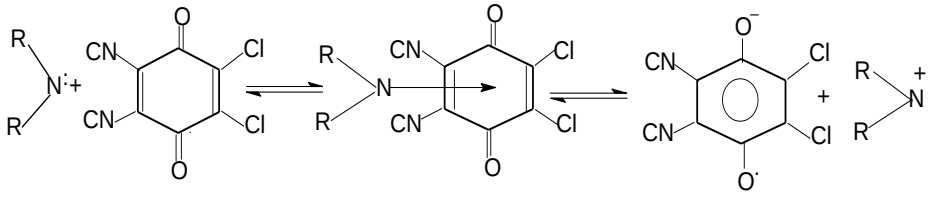
The interaction of drug with DDQ at room temperature was found to yield a colored charge transfer complex. In polar solvents, complete electron transfer from drug as an electron donor (D), to acceptor moiety (A) takes place resulting in the formation of intensely colored radical anion of DDQ. The reaction sequence can be shown in Scheme 2.



Drug (Donor) Acceptor (DDQ) Donor-acceptor complex or Charge transfer complex anion cation

Table 1: Spectraland analytical parameters of ion pair complexes of DDQ with drugs.

Parameter	Ast	Dom	Eso	Los	Sum	Ter
λ_{max} (nm)	545	545	545	545	545	545
Beer's law limit (μgml^{-1})	5 – 140	4 – 76	4 – 84	5 – 150	5 – 105	5.0 – 105
Molar absorptiv ($L\ mol^{-1}\ cm^{-1}$)	3750	3300	5100	2375	2440	3750
Formation cons K, M^{-1}	3800±60	3150±60	9200±50	2400±70	2300±70	4000±50
Sandell sensitiv ($\mu g\ cm^{-2}$)	0.1107	0.1049	0.069	0.1872	0.1229	0.1096
Slope b	0.0090	0.0095	0.0145	0.00534	0.00813	0.00913
Intercept (a)	0.0136	0.0079	0.00686	0.0092	0.0093	0.00994
Correlation coe	0.9989	0.9993	0.9998	0.9987	0.9998	0.9984
Standard deviat of intercepts (%	0.0039	0.0005	0.00242	0.00267	0.0027	0.00283
Limit of detecti μgml^{-1}	1.4396	0.1765	0.551	1.6531	1.097	1.0252
Limit of quantif μgml^{-1}	4.3189	0.5296	1.652	4.9593	3.292	3.0757
Reegression eq Y= bx+a	Y = 0.0136+ 0.0 x is Con.(μgml^{-1})	Y = 0.0079 + 0.0 x is Conc.(μgm	Y = 0.00686+ 0. x is Con.(μgml^{-1})	Y = 0.0092+ 0.0 x is Con.(μgml^{-1})	Y = 0.0093 + 0.0 x is Con.(μgml^{-1})	Y = 0.00994 + 0 x is Con .(μgml^{-1})

PROCEDURE FOR CALIBRATION

Six drugs are found to respond to DDQ in acetonitrile *viz.,* Esomeprazole, Terazosin, Astemizole, Domperidone, Losartan, Sumatriptan.

Different volumes of standard solutions of drug were transferred into a series of 10ml volumetric flasks. To each flask, 2ml of (2.6×10^{-3} *M*) DDQ solution in acetonitrile was added

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and remaining volume was made up by solvent. The absorbance of the solution was measured after 2 min. of mixing against blank at 455, 545, and 588nm.

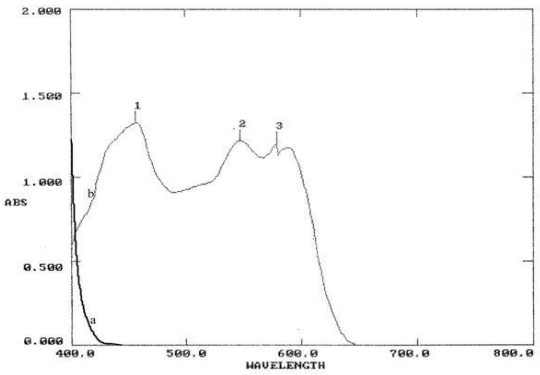

Figure 1: Absorption spectra of a) pure drug, b) DDQ in acetonitrile and c) its charge transfer complex with Astemizole.

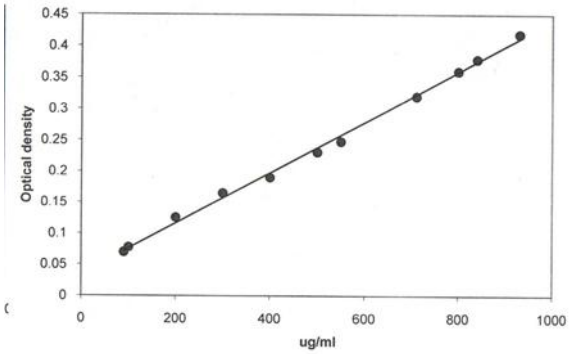

Figure 2: Calibration curve for quantification of Esomeprazole using DDQ as analytical reagent.

Calibration curves (Fig. 2) were linear for all the drugs whose limits are mentioned in Tables 32-37. Slope , intercept, correlation coefficient of the calibration curves are calculated and tabulated.

OPTIMIZATION OF FACTORS AFFECTING THE ABSORBANCE

Effect of concentration of acceptor

To establish the optimum concentration of reagent, Esopmeprazole 85 µg/ml, Terazosin 105 µg/ml, Astemizole 140 µg/ml, Domperidone 80 µg/ml, Losartan 150 µg/ml and Sumatriptan 105µg/ml were allowed to react with different volumes of *viz*., 0.4, 0.8, 1.2, 1.6, 2.0, 2.4ml of DDQ (2.6×10^{-3} *M*). The results showed that the highest absorbance was obtained with 1.8ml which remained unaffected by further addition of DDQ. Hence 2ml of the reagent was used for the determination of drugs (Fig. 3).

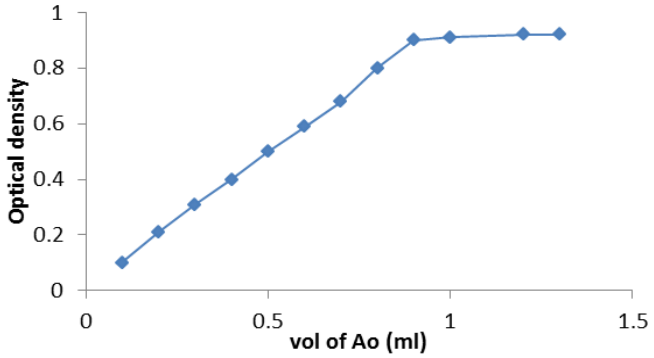

Figure 3: Effect of volume of reagent on the optical density of the Ion - pair complex of DDQ and Esomeprazole.

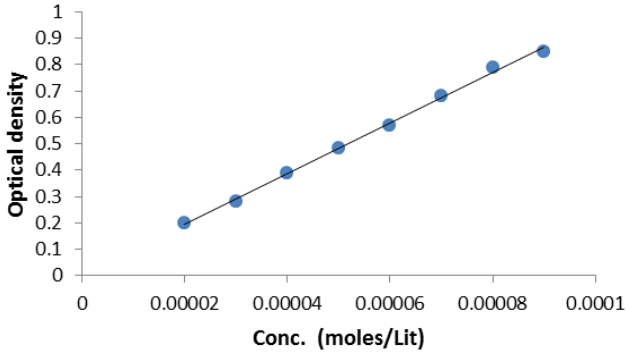

Figure 4: Determination of molar absorption coefficient of DDQ anion.

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The concentration of the drugs used in the study was chosen from random studies of effect of the concentration of the drug.

Effect of concentration of drug

To a fixed volume of acceptor mentioned above, different volumes of drug of random concentration were added. Solutions developed coloration. Absorbance was measured at 455, 545, and 588nm. Beer's law was obeyed by these solutions to certain extent of concentration and above which linearity was not observed. This concentration is taken as optimum concentration and stock was prepared. The stock was further diluted to get atleast 8 – 10 points in calibration curves range.

Similarly when the concentration is below certain limit, points scattered. This was taken roughly as a measure of limit of detection which is further confirmed by following the procedure for determination of LOD and LOQ.

Effect of reaction time

The interaction of DDQ with drugs resulted in the formation of colored product, which stabilized within 2 minutes of mixing. The developed color remained stable at room temperature for about an hour. After two hours many solutions turned brownish black and are opaque. After a day all solutions decolorized hence the measurements were made immediately after mixing the solutions.

Effect of Organic solvent

Both polar and non-polar solvents such as chloroform, methanol, 1,2- dichloroethane, acetone and acetonitrile were used to select elegant solvent for the analysis of the drug. Acetonitrile is found to be suitable solvent as it produces maximum optical density with a fixed concentration of drug, while other solvents mentioned above are found to be unsuitable as they produced lower absorbances due to incomplete dissociation of complex. Hence acetonitrile is used throughout the study (Table 2).

Table 2:The effect of solvent on the Optical density of charge transfer band of DDQ with Quetiapine (80 µg/ml).

s.no	Solvent	Optical density
1	Acetonitrile	0.84
2	Methanol	0.72
3	1,2- dichloroethane	0.15
4	Chloroform	0.13
5	Carbon tetra chloride	0.08

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VALIDATION OF THE PROPOSED METHODS

The methods developed have been validated in terms of guidelines of international conference of harminisation (ICH) [14] *viz.,* selectivity, sensitivity, precision, accuracy, linearity, LOD, LOQ Sandell's sensitivity and robustness. The methods are selective and can differentiate the analyte from the excipients.. 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. (Table 3). Sandell's sensitivity "Milligrams of drug per liter required to produce a change in the absorbance by 0.001 absorbance units" have been calculated for all the drugs. Limit of Detection "The lowest amount of analyte in a sample that can be detected, but not necessarily quantitated as an exact value" and Limit of Quantification " The lowest amount of analyte in a sample that can be quantified using Calibration curves" have been calculated by using equations available in the literature.

$$LOD = 3.3s/S$$
$$LOQ = 10s/S.$$

Where s= standard deviation of the intercept (n = 5)
S = slope of Calibration plot

The robustness of the methods are examined by performing the experiments on three different spectrophotometers with excellent tally of absorbance values. The methods developed have also been applied for the analysis of pharmaceuticals. The recovery experiments performed show high accuracy and precision and the results are compared to the available validated reported methods on each drug. The values %RSD and t- and F tests are in the permissible range of experimental errors. (Table 4) and show that the methods can be used in both pharmaceutical and drug industries

STABILITY CONSTANTS OF ION – PAIR CHARGE TRANSFER COMPLEXES

In literature the author noticed that Benesi - Hildebrand method (BH) [15] is widely used for determination of stability constant K and molar absorption coefficient ε.

$$Ao/ D = 1/ K (Do) \varepsilon + 1/ \varepsilon$$

Above equation is known as BH equation and a plot of Ao/ d Vs 1/ Do is a straight line from whose slope and intercept the *K* and ε are determined. The BH method however demands the concentration of donor Do > > Ao (Do should be 20 to 100 times the acceptor concentration) and many times the correct separation of *K* and εis also doubtful.

Many workers used the Benesi - Hildebrand method without fulfilling the condition Do > > Ao and the values of ε obtained varied widely. The ε reported [3,6] for DDQ : are 9×10^3. To 4.17×10^4.L $mol^{-1}cm^{-1}$

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It is surprising that the molar absorption coefficient of an ion which is expected to be constant and charecteristic of that ion is widely varied. Therefore it is thought worth to determine the molar absorption coefficients of acceptor anions and then use the values to determine the stability constant *K.* To accomplish this, different volumes of dilute solutions of *DDQ were* transfered to 25ml standard volumetric flask and excess drug was added and optical density was noted. The addition of drug continued until there is no appreciable increase in the optical density. A plot of d Vs concentration of acceptor gave a straight line from whose slope the molar absorption coefficient of anion of DDQ was determined (Fig 4). This experiment was repeated at least with three drugs and each experiment was repeated three to four times until constant value of molar absorption coefficient (9650 L $mol^{-1}cm^{-1}$) was observed. The stability constant *K* Is calculated using the molar extinction coefficient obtained from above experiment.

$$K = (d / \varepsilon) / [(Ao - (d / \varepsilon)] [Do - (d / \varepsilon)]$$

The stoichiometry of each of the complex has been determined from Job's continous variation method and found to be 1:1 in each case. A typical Job's plot of DDQ with Dextromethorphan is presented in (Fig.5)

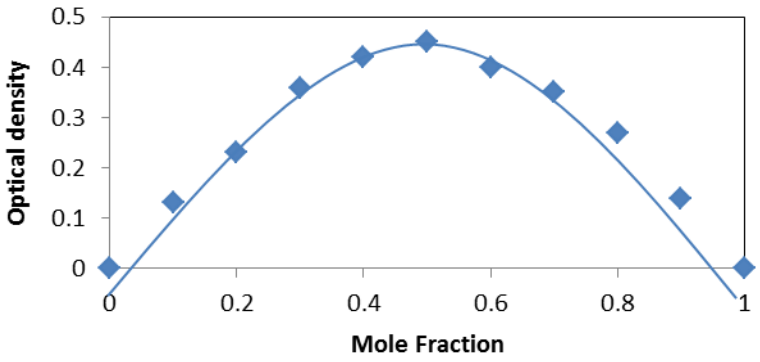

Figure 5: Job's Continuous variation plot of DDQ-Esomeprazole.

Structure activity relation

From the slopes of calibration curves and from formation constants it is clear that the donor abilities of the drugs are in the order : Esomeprazole > Terazosin > Astemizole > Domperidone > Losartan > Sumatriptan.

From the structures of the drugs, it is clear that Esomeprazole is a 2^{o} amine hence is expected to show highest basic character which is also in accordance with the slopes of Beer's law plots. Terazosin is a 1^{o} amine hence stands next to the 2^{o} amines in the basicity order. Astemizole, Domperidone, Losartan and Sumatriptan are 3^{o} amines and are found in the order and their basicities are next to the basicities of 2^{o} and 1^{o} amines.

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