Spectrophotometric Determination of drugs in bulk and pharmaceutical dosage forms by using Tetracyanoethylene

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ABSTRACT

A selective ,sensitive ,accurate UV-Visible spectrophotometric methods have been developed for the estimation of drugs *viz.*,Diflunisal (DFL),Febuxostat(FBT),Metaxalone(MTX),Fexofenadine methyl ester(FME) and Linezolid(LZD) in bulk and their pharmaceutical dosage forms using Tetracyanoethylene (TCNE) as analytical reagent. These method are based on the formation of charge transfer complexes of drugs as n-electron donor with TCNE as π-acceptor .The selected drugs turned the colorless solution of TCNE in Acetonytrile to yellow and exhibited a doublet at 400 & 420nm due to the formation of Complex of drugs with TCNE.Under the optimized experimental conditions ,Beer' s law is obeyed over the concentration ranges of 10-50 µg/ml ,5-25 µg/ml,15-75 µg/ml,5-25 µg/ml and 5-25 µg/ml for DFL,FBT,MTX,FME and LZD respectively. The effect of reagent concentrations, polarity of solvents and effect of reaction time have been studied and optimized. These methods have been validated in terms of ICH guidelines and applied to the quantification of selected drugs in bulk and dosage forms.

Key words: Drugs, Tetracyanoethylene, charge transfer complexes, spectrophotometry, and validation.

INTRODUCTION

Diflunisal: Diflunisal (DFL) is a salicylic acid derivative [1] and chemically is (2', 4'-difluoro-4-hydroxybiphenyl-3-carboxylicacid)]. It belongs to the class of non-steroidal anti-inflammatory drugs (NSAIDs) with analgesic, anti-inflammatory and antipyretic properties [2].

Through survey of literature reveals that a few analytical methods have been reported for estimation of Diflunisal *viz.* , by direct spectrophotometric method[3] Also DFL has been determined in different combinations by Spectrophotometric [4], multivariate calibration [5], spectroflurimetric [6,7],TLC-Densitometric [8]), HPLC[9,10], capillary electrophoretic [11] and electrochemical [12-14]methods.

Febuxostat: Febuxostat is a novel xanthine oxidase inhibitor indicated for the chronic management Of hyperuricemia in patients with gout [15], the active ingredient in ULORIC (febuxostat) is 2-[3-Cyano-4-(2-methylpropoxy) phenyl]-4-methylthiazole-5-carboxylic acid [16] Febuxostat is a non purine analogue inhibitor of both the oxidized and reduced forms of xanthine oxidase [17].

A few spectrophotometric, HPLC, LC-MS, TLC and GC methods were reported earlier for the determination of Febuxostat in bulk and pharmaceutical dosage forms [17].

Metaxalone: Metaxalone chemically known as 5-[(3, 5-dimethylphenoxy) methyl]-1, 3-oxazolidine-2-one. It is a centrally acting muscle relaxant, used to relax muscles and relieve pain caused due to strain, sprains [18]. Metaxalone belongs to non benzodiazepine antispasmodics with a structure similar to mephenaxalone nucleus. It acts through inhibiting interneuronal activity and blocking polysynaptic reflex pathways at spinal cord and at descending reticular formation in brain but leaving monosynaptic pathways intact like other similar class of skeletal muscle relaxants [19].

Literature survey revealed that there are few methods reported for the determination of metaxalone in plasma by liquid chromatography soft ionization interfaces like electro spray ionization (ESI) ,ultraviolet spectroscopy with LC Chromatography (HPLC-UV) , UV spectroscopy , gas chromatography with flame ionization detection and gas chromatography with mass detection [20].

Fexofenadine methyl ester: Fexofenadine, α, α-dimethyl-4-[1-hydroxy-4-[4-(hydroxydiphenyl-methyl)-1-piperidinyl] butyl]-benzene acetic acid[21] , Fexofenadine is a histamine H1-receptor antagonist used therapeutically for the treatment of allergic rhinitis and chronic idiopathic urticaria [1]; It is given orally in doses ranging from 30 to 180 mg/day. Fexofenadine is predominantly eliminated unchanged in bile (80%) and urine (11%); approximately 5% is metabolized to methyl ester (3.6%) and azacyclonol (1.5%) metabolites [22].

A few methods like ultraviolet, fluorescence detection, and many methods for determination of fexofenadine in human plasma based on HPLC with mass spectrometric detection or tandem mass spectrometric detection [23]. Sample processing for almost all of the methods reported use costly solid phase extraction (SPE) with C18 cartridges or Oasis TM HLB cartridges [24].

Linezolid: Linezolide (LNZ) chemically, (s)-N-[[3-[3-fluoro-4(4-morpholinyl) phenyl]-2-oxo-5-azolidinyl] methyl] acetamide [25], is an oxazolidinone antibiotic used for the treatment of serious infections caused by Gram-positive bacteria, including vancomycin-resistant enterococci (VRE) and methicillin-resistant Staphylococcus aureus (MRSA) [26].

The methods reported in the literature for determination of linezolid include UV spectrophotometry, HPLC using UV-detection and fluorescence detection, capillary electrophoresis, TLC followed by densitometric analysis and microbiological assay [27–33].

Thorough survey of literature on the selected drugs revealed that quantification using *TCNE* as analytical reagent has not been reported yet although the reagent is common, known to offer simple, sensitive method of quantification for drugs. This paper reports simple, direct, and sensitive spectrophotometric method for determination of drugs using TCNE as π-acceptor based on the formation of charge transfer complex.

EXPERIMENTAL

Instrument: Shimadzu 2600 double beam UV-Visible spectrophotometer is used to record the spectra of individual components as well as the charge transfer complexes, using matched pair of Quartz cells of 10mm path length.

Materials: The Tetracyanoethylene is supplied by sigma Aldrich .The AR grade solvent acetonitrile and methanol are supplied by SD Fine chem...Ltd.Mombai, India. The drugs used in study are procured from Hetero drugs pvt.ltd. Hyderabad.

PREPARATION OF STANDARD STOCK SOLUTION

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

RESULTS AND DISCUSSION

The TCNE solution of 0.5mg/ml in acetonitrile was freshly prepared. Aliquots of drugs (0.5-2.5) were transferred into a series of 10ml calibrated flasks, to each flask, 1.5 ml of TCNE solution in acetonitrile was added and remaining volume was made up by solvent. The absorbance of yellow colored solution was recorded after 20min of mixing against reagent blank at 400nm and 420 are plotted against the corresponding concentrations (µg/ml) of the drug to construct the calibration curve.

DETERMINATION OF DRUGS IN DOSAGE FORM

Ten tablets were weighed, finely powdered and an accurately weighed quantity of the powdered tablet contents equivalent to 50mg of the active ingredient was transferred into a 50ml 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 on water bath for the complete dissolution of drug. 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, Diflunisal 50 µg/ml ,Febuxostat 25 µg/ml, Metaxalone 75 µg/ml, Fexofenadine methyl ester25 µg/ml and Linezolid 25 µg/ml were react with different volumes of TCNE (3.9×10^{-3}) .The results showed that the highest absorbance was obtained with 1.5ml .Hence 1.5ml of reagent was used for the determination of drugs.

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 TCNE it produces maximum absorbance with a fixed concentration of drugs, while other solvents produced lower absorbance due to incomplete dissociation of complex.

EFFECT OF REACTION TIME

The interaction of TCNE with drugs resulted in the formation of ion- pair complexes which stabilized with in 20 min of mixing. The developed color remained stable at room temperature for about an hour. After a day all solutions are decolorized.

VALIDATION OF THE PROPOSED METHOD

The methods developed have been validated in terms of guidelines of international conference of harmonization *viz.*, selectivity, sensitivity, precision, accuracy, linearity, LOD, LOQ. Sandell's sensitivity and

robustness. The precision is tested by repeating each experiment atleast 6 times while the accuracy has been tested by taking known weight of sample and performing recovery experiments. The robustness of the method was examined by performing the experiments on three 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 (Table 2).And show that the methods can be used in both pharmaceutical and drug industries.

STABILITY CONSTANTS OF CHARGE TRANSFER COMPLEXS

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

$A_0/d = 1/K (D_0) € + 1/€$

Where A_0 = conc. of acceptor, d= optical density, D_0 = conc. of drug, €=Molar absorption coefficient and K= stability constant.

A plot of A_0/d Vs $1/D_{0\ is}$ a straight line from whose slope and intercept the K and € are determined.

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 TCNE is presented in (Fig-6).

CONCLUSION

TCNE forms charge transfer complexes with selected drugs and exhibits doublet at 400nm and 420nm.The interaction enabled the quantitative determination of these drugs. This method is validated in terms of precision, accuracy, linearity and robustness; conditions are optimized and applied to the analysis of pure drug and pharmaceutical dosage forms.

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Table [1]: Spectral, analytical and statistical parameters of charge transfer complexes of drugs with TCNE.

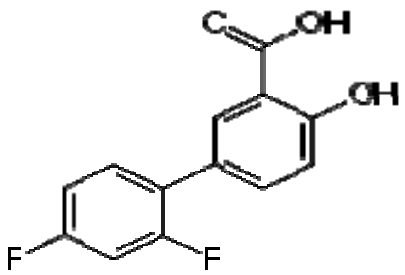
Drugs name parameters	Diflunisal	Febuxostat	Metaxalone	Fexofenadine methyl ester	Linezolid
λ max, nm	400 & 420	400 & 420	400 & 420	400 & 420	400 & 420
Beer's law limit (μg/ml)	10-50	5-25	15-75	5-25	5-25
Molar absorptivity ($L\ mol^{-1}\ cm^{-1}$)	12900	16333	9013	25720	15800
Slope(specific absorptivity),b	0.0096	0.0085	0.0024	0.0219	0.004
Intercept, a	0.0704	0.1167	0.1009	0.0961	0.06
Correlation coefficient, r	0.9987	0.9992	0.9988	0.9989	0.999
Sandell's sensitivity ($\mu g\ cm^{-2}$)	0.104	0.1176	0.4166	0.0456	0.25
Formation constant, $K(M^{-1})$	1010	1124	839	2593	321
Standard deviation of intercepts (n=6)	0.0016	0.00148	0.0029	0.0012	0.0017
Limit of detection (μg/ml)	0.55	0.5435	3.9875	0.1808	1.4025
Limit of quantification (μg/ml)	1.6666	1.6471	12.0833	0.5479	064.25
Regression equation y=a+bx; x=concentration of drug(μg/ml)	0.0704 + 0.0096	0.1167 + 0.0085	0.1009 + 0.0024	0.0961 + 0.0219	0.06 + 0.004

Table [2]: Application of proposed method for the analysis of the studied drug in their pure form.

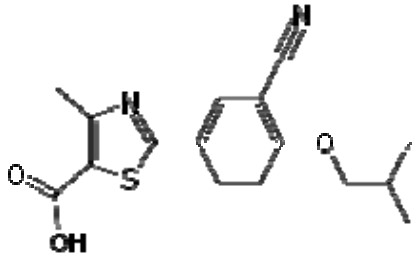
	Diflunisal	Febuxostat	Metaxalone	Fexofenadine methyl ester	Linezolid
Amount taken (µg/ml)	10 20 30 40	5 10 15 20	15 30 45 60	5 10 15 20	5 10 15 20
Amount Found (µg/ml)	9.99 20.01 29.98 39.99	4.98 9.99 15.02 19.99	14.95 29.98 45.02 59.94	4.98 9.98 14.99 19.97	4.98 10 14.97 19.99
% Recovery	99.9 100.05 99.93 99.97	99.6 99.9 100.13 99.95	99.66 99.93 100.04 99.9	99.6 99.8 99.93 99.85	99.6 100 99.8 99.95
% RSD	0.06 0.10 0.15 0.21	0.13 0.22 0.11 0.25	0.16 0.33 0.25 0.21	0.14 0.06 0.12 0.15	0.18 0.14 0.09 0.21
Proposed Mean ± SD	99.96 ± 0.06	99.89 ± 0.2201	99.88 ± 0.1607	99.79 ± 0.1405	99.83 ± 0.179
Ref Mean ± SD	100.64 ± 1.85	99.22 ± 0.64	99.66 ± 0.14	99.4 ± 0.49	101.1 ± 1.55
t-test	1.2782	1.3087		1.0596	3.7219
F-test	0.001	0.1181	1.3163	0.0820	0.0133

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

	Diflunisal	Febuxostat	Metaxalone	Fexofenadine methyl ester	Linezolid
Amount taken (µg/ml)	20 30 40 50	10 15 20 25	30 45 60 75	10 15 20 25	10 15 20 25
Amount Found (µg/ml)	19.98 29.99 40.03 49.87	9.99 14.97 19.99 25.01	29.96 44.99 59.97 75.08	10.05 14.98 19.96 25.04	9.99 15.1 19.97 24.98
% Recovery	99.9 99.96 100.07 99.74	99.9 99.8 99.95 100.04	99.86 99.97 99.95 100.1	100.5 99.86 99.8 100.16	99.9 100.66 99.85 99.92
% RSD	0.141 0.137 0.075 0.089	0.074 0.118 0.087 0.206	0.067 0.043 0.167 0.213	0.297 0.449 0178 0.247	0.367 0.426 0.066 0.188
Proposed Mean ± SD	99.91 ± 0.1376	99.92 ± 0.1001	99.97 ± 0.0989	100.08 ± 0.3212	100.08 ± 0.3861
Ref Mean ± SD	100.44 ± 1.685	99.5 ± 0.50	100.14 ± 0.11	103.26 ± 2.857	100.1 ± 1.63
t-test	4.0873	1.1538	0.5842	2.1618	1.8188
F-test	0.0066	0.04	0.8016	0.0126	0.0561



Diflunisal



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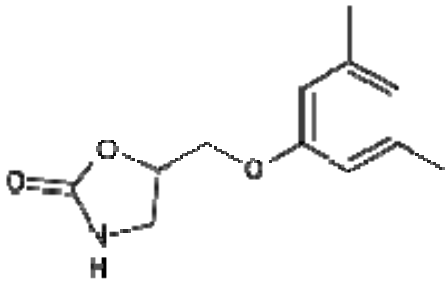

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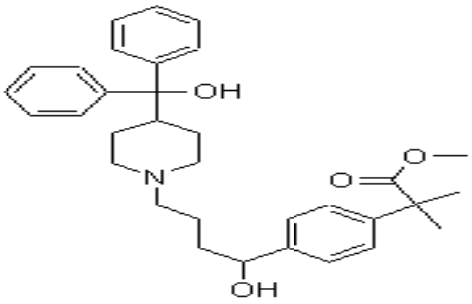
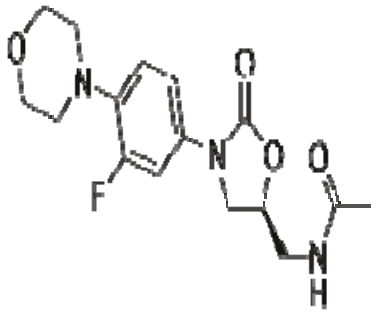
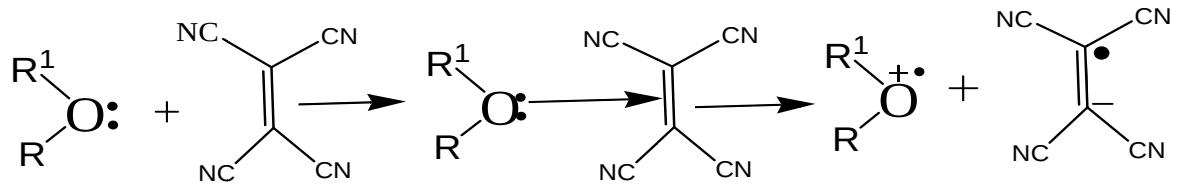


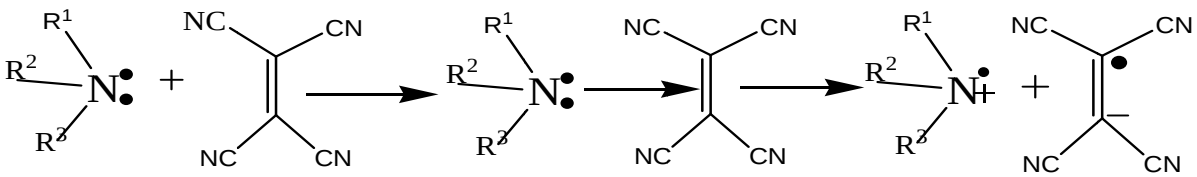
Fexofenadine methyl ester



Linezolid



Diflunisal TCNE Charge transfer complex anion cation



Drug s TCNE Charge transfer complex anion cation

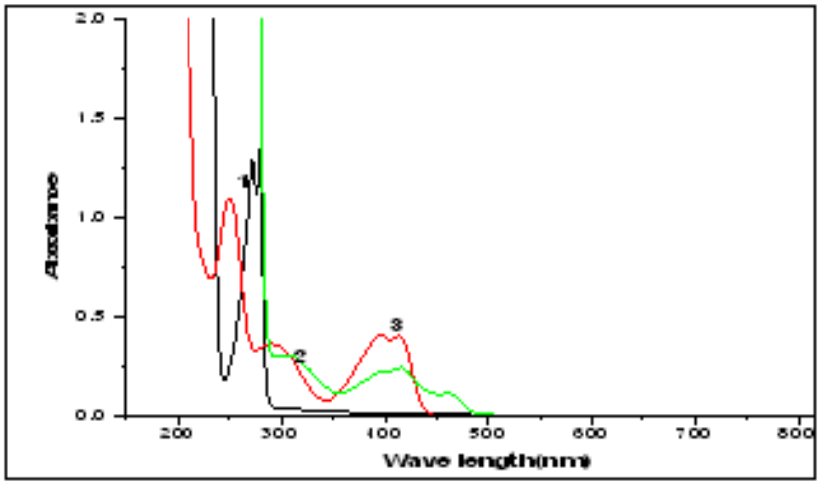


Fig (1) :(1) Metaxalone in Acetonitrile, (2) TCNE in Acetonitrile and (3) Charge transfer complex of Metaxalone with TCNE.

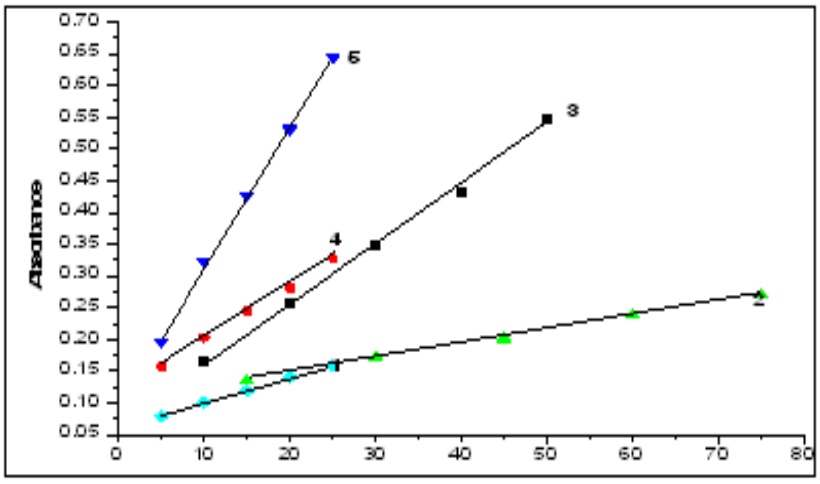


Fig (2): Calibration curves of TCNE with (1) Linezolid,(2) Metaxalone,(3) Diflunisal,(4)Febuxostat and (5) Fexofenadine methyl ester.

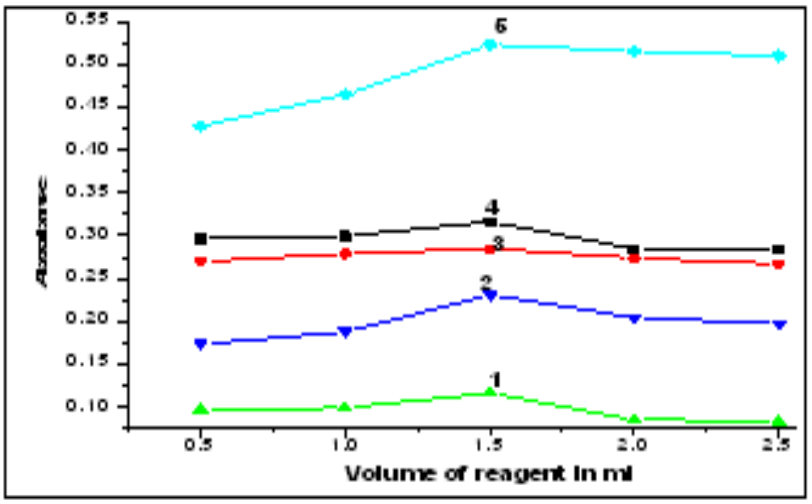


Fig (3): Effect of volume of reagent on the optical density of the Ion - pair complex of *TCNE* and Metaxlone (1), Fexofenadine methyl ester (2), Febuxostat (3), Diflunisal (4) and Limuloid (5).

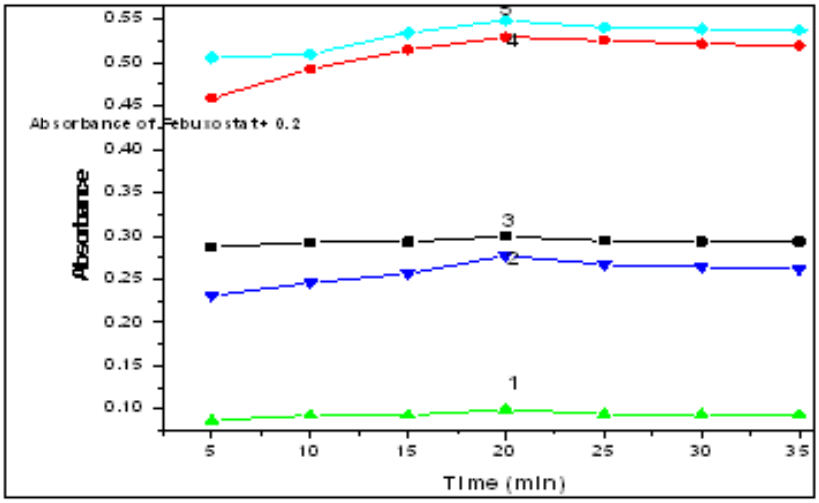


Fig (4): Effect of reaction time on formation of charge transfer complexes of TCNE and Metaxlone (1), Fexofenadine methyl ester (2), Diflunisal (3), Febuxostat (4) and Linelozid (5).

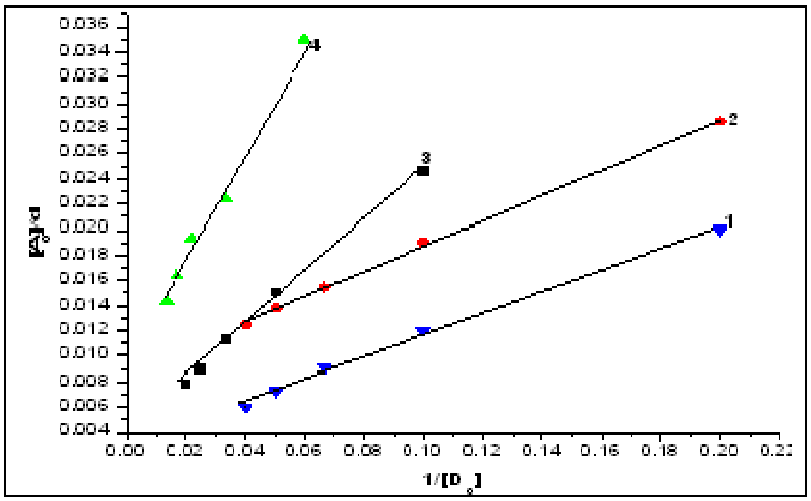


Fig (5): Benesi – Hildebrand plot of TCNE with (1) Fexofenadine methyl ester, (2) Febuxastat, (3) Diflunisal and (4) Metaxalone.

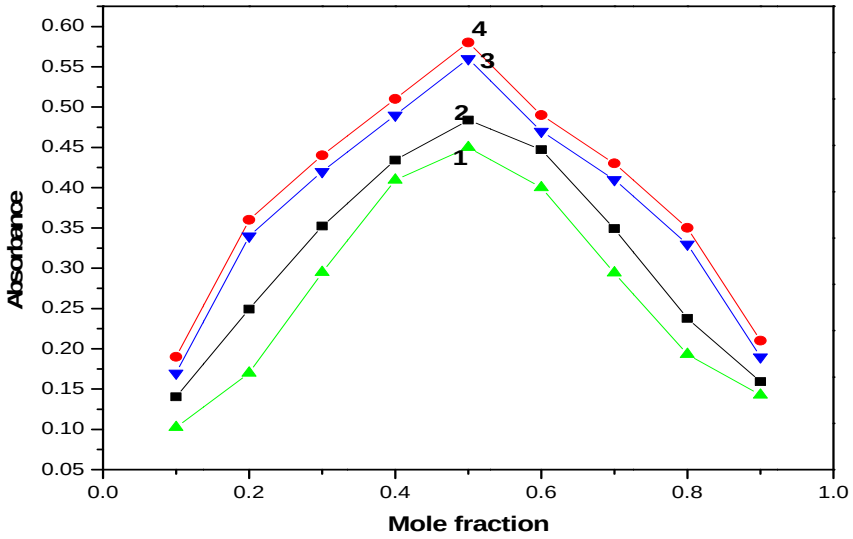


Fig (6): Job`s continuous variation plot of TCNE with (1) Linezolid,(2)Diflunisal,(3) Febuxostat and(4) Fexofenadine methyl ester