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QUANTIFICATION OF DRUGS USING TETRACYANOETHYLENE AS ANALYTICAL REAGENT

T. Charan Singh*

G. Narayanamma Institute of Technology and Science Shaikpet, Hyderbad.

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***Corresponding Author**
Dr. T. Charan Singh
G.Narayanamma Institute of Technology and Science Shaikpet, Hyderbad.

ABSTRACT

Five Drugs viz. Esomeprazole, Losartan, Paroxetine, Rabeprazole, Tamoxifen were tested for the formation of charge transfer complexes with Tetracyanoethylene(TCNE). Each of these drugs turned the colourless reagent i.e. TCNE., in CH_3CN, to pale yellow and exhibited two bands at 400 and 420nm due to anion of the reagent the intensity of these bands increased with increase in the concentration of the drugs and formed a bases 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 effects of reagent concentration, polarity of solvent, and interference of excipients have been studied & optimised the 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 variations of slopes of calibration plots and stability constant of the complexes are discussed in terms of structures of drugs.

KEYWORDS: TCNE, Esomeprazole, Losartan, Paroxetine, Rabeprazole, Tamoxifen, Quantification.

1. INTRODUCTION

Tetracyanoethylene (TCNE) is known for its interaction with drugs having donor sites in their structures and from ion-pair charge transfer complexes which offers basis for quantification of drugs.[1-3]

Through survey of literature on the following 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 of drugs. This prompted the authors to

develop quantification methods for the following drugs, (Scheme 1), using TCNE as a chromogen and hence tested them for the formation of charge transfer complexes which is accepted to form a basis for the quantification of the drugs the physiological activity of drugs and methods used so far for their quantifications are:

1.1 Esomeprazole

Esomeprazole magnesium (Fig. 1) 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 a tetracyclic achiral cationic sulphenamide. This then reacts with specific cysteines resulting in the inhibition of the H^+/ K^+ - APTase enzyme.[4]

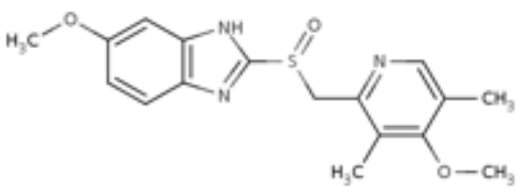


Fig.1 Esomeprazole 6-methoxy-2-[(4-methoxy-3, 5-dimethylpyridin-2-yl)methylsulfinyl]-1H-benzimidazole

Esomeprazole magnesium has been studied and determined by several procedures such as spectrophotometric methods [5-10], differential scanning calorimetry.[11], High Performance Liquid Chromatography.[12-14], Capillary Electrophoresis.[15], Reverse phase liquid chromatography [16], Liquid chromatography with tendem mass spectrometry.[17- 18].

1.2 Losartan K

Losartan K (Fig. 2) belongs to the anti- hypertensive group of drugs known as angiotensin II receptor antagonist. It is used in the treatment of hypertension with heart failure or renal impairment by blocking the binding of angiotensin II to AT1 receptor found in vascular smooth muscles, Adrenaline glands, etc.

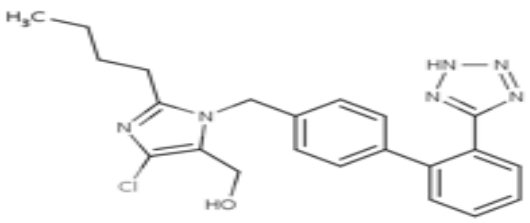


Fig. 2 Losartan K6-methoxy-2-[(4-methoxy-3,5-dimethylpyridin-2-yl)methylsulfinyl]-1H-benzimidazole

Various analytical techniques are reported for the estimation of Losartan such as High Performance Thin Layer Chromatography[19-20], High Performance Liquid Chromatography[21-24], Capillary Electrophoresis[25-26], Spectroscopy[27-29] and Kinetic spectrometry.[30-31]

1.3 Paroxetine: Paroxetine (Fig. 3) is a new generation anti depressant drug. It exerts its antidepressant effect through a selective inhibition for the reuptake of the neurotransmitter serotonin by the pre-synaptic receptors. It is also prescribed in the treatment of related disorders, such as obsessive – compulsive disorder, panic fits, social phobia and post trauma stress.

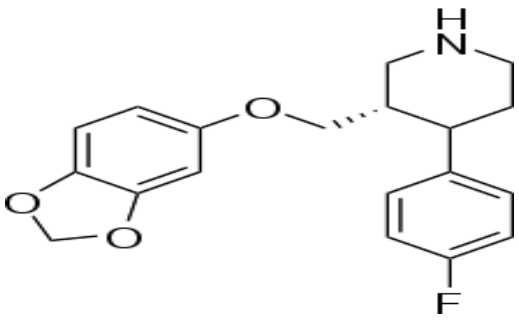


Fig. 3: Paroxetine (3S,4R)-3-[(2H-1,3-benzodioxol-5-yloxy) methyl]-4-(4-fluorophenyl)piperidine

The methods reported for its quantitative determination in dosage and or biological fluids include voltametry[32], densitometry[33], HPLC[34], gas chromatography[35] and spectrophotometry.[36]

1.4 Rabeprazole Na

Rabeprazole sodium (Fig.4) is a proton pump inhibitor that suppresses gastric acid secretion by specific inhibition of the enzyme system of hydrogen / potassium adenosine triphosphate (H+/ K+ATPase) at the secretary surface of the gastric parietal cell. It is indicated for the treatment or symptomatic relief of various gastric disorders gastroesophageal reflux disease and pathological hypersecretory conditions including Zollinger- Ellison syndrome. It is a substituted benzimidazole. Like most compounds of this class, it is decomposed in acid media to yield two main products, the sulfonamide and benzimidazole sulphide.

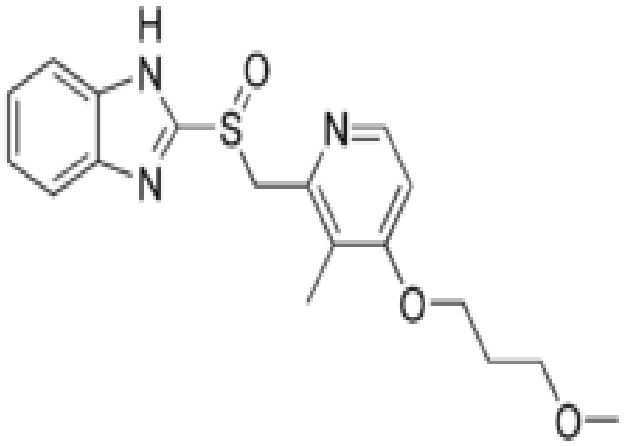


Fig. 4: Rabeprazole sodium2-([4-(3-methoxypropoxy)-3-methylpyridin-2-yl] methylsulfinyl)-1H- benzo[d]imidazole

A survey of the literature revealed very few methods for the determination of rabeprazole in pharmaceutical formulations or biological fluids. These methods include non- aquous capillary electrophoresis.[37], High Pressure Liquid Chromatography with NMR.[38], mass spectrophotometry.[39], ultraviolet spectrophotometry [40]. A differential pulse anodic volta metric method at a glassy carbon electrode was published for its determination in tablet dosage form.[41] and spectrophotometric and chromatographic determination.[42] was also reported.

1.5 Tamoxifen

Tamoxifen (Fig. 5) is a non-steroidal triphenyl ethylene derivative that inhibits the action of estrogens. It is a mixed estrogen agonist and antagonist which suppresses tumour growth. It is widely used in the treatment of hormone-sensitive breast cancer. Mechanism of action studies indicate that Tamoxifen citrate binds with estrogen receptors forming a Tam-17 beta-estradiol receptor complex which binds to the nuclear binding sites on the genome. Tamoxifen binds to cytoplasm estrogen receptors in tissue such as breast, anterior pituitary and prostrate tissue. It

exhibited pH dependent fungicidal activity (optimum pH 7.5) against yeast cell of calbicans.[42] It combined with vinblastine was cytotoxic to human prostate cancer cells.

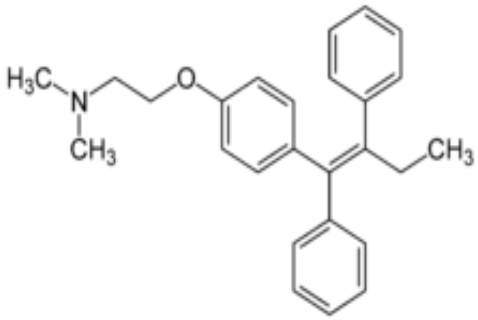


Fig. 5: Tamoxifen (Z)-2-[4-(1,2-diphenylbut-1-enyl)phenoxy]-N,N-dimethyl-ethanamine

Spectrophotometric methods for the determination of tamoxifen citrate (TC).[43] including the use of naphthalene Blue 12BR and Alizarine Red – S [44] have been reported. Methods for determination of the purity by Gas Chromatography, mass spectra, HPLC and TLC have been reported.[45-49] The GC-MS analysis of TC and its metabolites in plasma.[50] and the X- ray crystallographic structures of Tamxifen have also been reported.[51]

2 EXPERIMENTAL

2.1 Instrument

The spectra (fig.6) of individual components and charge transfer complexes were recorded on Shimadzu 140 double beam spectrophotometer as well as on thermo Nicolet 100 and Elico 159 UV- Visible single beam Spectrophotometer using matched pair of quartz cells of 10mm path length

2.2 MATERIALS

TCNE was obtained from SD fine chem. India Ltd. It was recrystallized thrice from chlorobenzene and vacuum sublimed to get pure white crystals of TCNE. A stock solution of 100mg/100ml (w/v) (7.812×10^{-3}M) in acetonytrile was freshly prepared, the drugs used in study or produced from Hetero Drugs Pvt Ltd. Hyderabad. Most of the drugs procured are in the form of their acid salts hence they have been neutralised by adding calculated amount of NaOH/ NH_4OH as required followed by extraction with either or $CHCl_3$. They were recrystallised 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 spectro grade acetonytrile, AR grade methanol, either NaOH and NH_4OH all of them are supplied by SD fine Chemicals, Mumbai.

Extraction of drugs for Pharmaeuceutical analysis

3.1 Esomeprazole

Twenty tablets were weighed, and finely powdered. An accurately weighed quantity of the powdered tablet contents equivalent to 50mg of the active ingredient was transferred into a 100ml calibrated flask, and dissolved in about 100ml of methanol. The contents of the flask were swirled, sonicated for 5minutes. The mixture was mixed well, filtered and evaporated to dryness. Residue was dissolved in acetonitrile heating on water bath for the complete dissolution of drug. A measured volume of the prepared solution was diluted quantitatively to 100ml with acetonitrile, and the resulting solution was used for the analysis.

3.2 Losartan

Two tablets (Losartan K-400mg) were powdered and equivalent amount of 200mg of ofloxacin was added to about 100ml of methanol and filtered through Whatmann filter paper. The residue was washed thrice with methanol for complete recovery of the drug and methanol was evaporated. To the content acetonitrile solvent is added. The aliquot portions of this stock solution were further diluted with solvent to get the final concentration required for the determination of the drug

3.3 Paroxetine

Ten tablets (Pexep-10mg) were weighed, powdered and an accurately weighed amount of powder equivalent to 50mg of paroxetine HCl was dissolved in 40ml of water followed by sonnication for 20minutes, then filtered into a volumetric flask. The residue was washed with a few millilitres of water and filtered, washings were added to the same flask. The paroxetine hydrochloride solution was taken in a separating funnel containing ether followed by the addition of 0.1 N sodium hydroxide. The contents are shaken well and allowed to stand for some time. Both the layers are separated. Extraction continued in two portions with 25ml of ether. Ethereal layer is separated and evaporated. To the content acetonitrile is added and further dilutions are done depending up on the requirement.

3.4 Rabeprazole

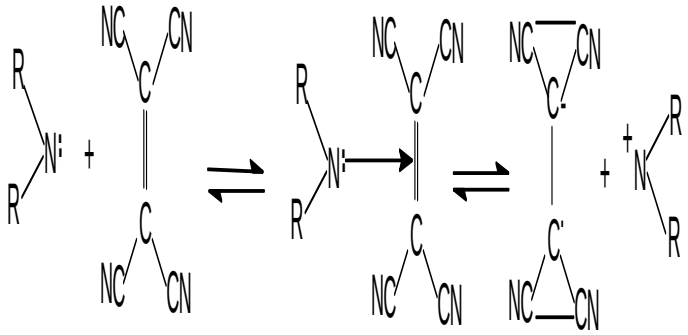
Twenty tablets (Razo-10mg) were weighed accurately and a quantity of tablet powder equivalent to 50mg was weighed and dissolved in the 50ml of methanol for 5minutes and volume was filtered through Whatmann filter paper in a beaker. The residue was washed with methanol for the complete recovery of the drug. Methanol was evaporated and the content

was dissolved in 1,2 dichloroethane followed by heating for complete dissolution of drug. The content was cooled and was serially diluted for analysis.

3.5 Tamoxifen

Batches of ten tablets (cytotam-10mg) 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 and then dissolved in sufficient volume of water and filtered with whatmann filter paper. The residue was washed with a few millilitres of water. The filtrate is taken in separating funnel containing 1,2 dichloroethane (in case of iodine acceptor) and 0.1 N NaOH solution is added for neutralisation. The content was shaken for 5minutes. Organic layer get separated and extraction continued in two portions with 25ml of required solvent. Required solvent is added to the solution and diluted accordingly. In case of TCNE and p-CA 1,2 dichloroethane was completely evaporated and residue was dissolved in acetonitrile.

The proposed mechanism Scheme-1



Donor-acceptor complex or Charge transfer complex formation

4. SPECTRA

The spectra of ion-pair Charge transfer complexes were record in CH_3CN for quantification studies as well as to evaluation 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 note on fixed mode.

5. RESULTS AND DISCUSSION

Tetracynoethylene (TCNE) is a ‘’π” acceptor. It shows an absorption maximum at 280nm. When the colourless solution of donor is added to the solution of TCNE in Acetonitrile, it

turned yellow and exhibited bands at 400 and 420nm as doublet, characteristic of TCNE anion radical. From literature it is noticed that the radical formation was attributed due to the dissociation of charge transfer complex with a complete one electron transfer from the drug donor to TCNE acceptor. The dissociation of donor- acceptor complex is promoted by the high ionizing power of the solvent, *i.e.* Acetonitrile. The intensities of the bands at 400 and 420nm were found to be linearly related to the concentration of drug. The same observation forms a basis and has been applied to the variety of drugs of their quantitative analysis.

6. PROCEDURE FOR CALIBRATION

Five drugs *viz*, Esomeprazole, Losartan, Paroxetine, Rabeprazole and Tamoxifen showed colour changes when mixed with TCNE in CH_3CN. The changed colour is characteristic of TCNE anion and showed a double band at 400 and 420nm.

Accurately weighed aliquots of above mentioned in the concentration range shown in table were transferred in to a series of 10 ml volumetric flasks followed by 1 ml acetoniltrile. The spectra of each of the drug solution was scanned from 200-80nm against blank for 2 or 3 different concentrated samples as typical and absorbance was noted at 400 and 420nm for remaining samples in fixed mode. Each drug sample is analysed minimum of five times. All parameters are calculated and tabulated in Table .1 .

Calibration curves are plotted for all the drugs and found to be linear with small intercepts (fig.7). All other parameters such as slopes, correlation coefficients LOD, LOQ, Standard deviations are tabulated in respective table.1.

7. OPTIMIZATION OF FACTORS AFFECTING THE ABSORBANCE

7.1 Effect of concentration of reagent

The optimum reagent concentration was determined by adding various volumes of TCNE to a fixed concentration of 50µg/ml of Esomeprazole, 70µg/ml of Losartan, 35µg/ml of Paroxetine, 50µg/ml of Rabeprazole, 60µg/ml of Tamoxifen. It was found that 0.8 ml of in Acetonitrile for all the drugs were enough to develop the absorbance to its maximum intensity. 0.8ml of TCNE is optimum and larger volume had no effect on the absorbance of the coloured species. Therefore an excess of reagent *i.e.* 1ml of reagent in a total volume of 10 ml of reaction mixture was used throughout the work**.**

7.2 Effect of concentration of drug

Effect of concentration of drug has been checked. The optimum values of the concentration of drugs under study are mentioned in the table-1.

7.3 Effect of time

The reaction time determined by following the absorbance of developed color at different time intervals at ambient temperature (25±0.5^{o}C). Complete color development was attained instantaneously with all compounds investigated and the color remains stable for at least for 10-30 minutes.

7.4 Effect of organic solvent

Several solvents like Acetonitrile, Chloroform, Carbon tetrachloride, 1, 2-Dichloroethane and Methanol were used but found unsuitable for the analysis of the drugs. Polar solvent i.e Acetonitrile is found to be elegant solvent in the analysis of drugs with TCNE as it gives maximum absorbance due to complete dissociation of the complex.

8. VALIDATION OF THE PROPOSED METHODS

The methods developed have been validated in terms of guidelines of international conference of harminisation (ICH)[11] viz, selectivity, sensitivity, precision, accuracy, linearity LOD < LOQ Sandell's sensitivity and robustensess. The methods are selective and can differetitate 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 weight of sample and performing recovery experiments. The values % RSD and t-and F tests are in the permissible range of experimental errors (Table.2). Sandell's sensitivity "Milligramsof drug per liter required to produce a change in the absobance by 0.001 absorbance units" have been calculated for all the drugs. Limit of Detecton "The lowest amount of analyte in as ample that can be detected, but not necessarily quantitated as an exact value" and Limit of Quantificaiton" The Lowest amount of analyte in asample that can be quantified using Calibration curves"have been calculate 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 thee 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.2) And show that the methods can be used in both pharmaceutical and drug industries.

9. STABILITY CONSTANTS OF ION – PAIR CHARGE TRANSFER COMPLEXES

In literature the author noticed that Benesi – Hildebrand method (BH)[12] is widely used for determination of stability constant K and absorption coefficient, ε.

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

Above equation is known as BH eqation 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 acceptor concentration) and many times the correct separation of K and ε is also doubtful. Many works used the Benesi – Hildebrand method without fulfilling the condition Do >> Ao and the values of ε obtained varied widely. The ε reported for TCNE: are $9x\ 10^{3}$ to $4.17x\ 10^{4}$lit mol^{-1} $cm\text{-}^{1}$.

It is surprising that the molar absorption coefficient of an ion which is expected to be constant and characteristic of that ion is widely varied. Therefore it is thought worth to determine the molar absorption coefficients of acceptor anions and then use values to determine the stability constant *K*. To accomplish this, different volumes of dilute solution Of TCNE were transferred to 25ml standard volumetric flask and 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 TCNE was determined 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 (30,150lit $mole^{-1}$ cm^{-1}) was observed. The formation constants (K) have been determined for all drugs by taking different volumes of TCNE ($2.97x10^{-4}$ m) in Acetonitrile and followed the procedure as discussed. The concentration of TCNE vs. d gave a straight line from whose slope determined (Fig.8).

The shapes of the calibration curves and the formation constants are in accordance with the expectation.

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

Is calculated using the molar extinction coefficient obtained from above experiment?

The stioichiometry of each of the complex has been determined from job's continuous variation method and found to be1:1 in each case. A typical job's plot of TCNE with drug Esomeprazole is presented in fig.9

10. Structure activity relation

From the slopes of calibration it is clear that the donor abilities of the drug are in the order: Esomeprazole > Paroxetine > Tamoxifen > Rabeprazole > Losartan. From the structures of Drugs the order of the basicity is Esomeprazole and Tamoxifen are greater than Rabeprazole, Paroxetine greater than Losartan.

11. Application of the proposed methods for the analysis of Tablets.

As mentioned tablets of each drug was used to prepare solutions required for the spectral analysis. Each drug solutions was analysed using 4 to 5 samples each in the calibration range of corresponding drug. The calibrations curves are used to find the concentration of prepared tablets solution. Amount taken, Amount percentage recovery and parameters are presented in Table.3

Spectral and analytical parameters of ion pair complexes of TCNE with drugs Table 1.

Parameter	Esomeprazole	Losartan K	Paroxetine	Rabeprazole	Tamoxifen
λ_{max}(nm)	420	420	420	420	420
Beer's law limits (μgml^{-1})	1.5-32	2.5-19	4-32	5.5-40	4.5-32
Molar absorptive (L mol-1 cm-1)	11260	14740	7690	6790	8440
Formation constant,K,M^{-1}	750 ± 50	1320 ± 50	340 ± 10	360 ± 10	360 ± 10
Sandal sensitivity (µg cm-1)	0.0335	0.0214	0.0547	0.0554	0.0449
Slope b	0.02985	0.0416	0.0166	0.0164	0.0203

Intercept (a)	-0.00688	0.01075	0.00148	-0.0066	0.014
Correlation coefficient	0.9993	0.9963	0.996	0.9977	0.9951
Standard deviation of intercepts (%n=5)	0.0065	0.0021	0.00307	0.0052	0.00515
Limit of detection µgml^{-1}	0.783	0.2356	0.6081	1.3636	0.8118
Limit of quantification µgml^{-1}	2.1548	0.7187	1.8362	4.0969	2.4416
Regression equation Y=bx+a	Y=0.00168+0.0176x x is Conc (µgml-1)	Y=0.0102+0.0426x x is Conc (µgml-1)	Y=0.00164+0.0176x x is Conc (µgml-1)	Y=0.0074+0.0175x x is Conc (µgml-1)	Y=0.00164+0.0176x x is Conc (µgml-1)

Determination of accuracy and precision of the methods on pure drug samples Table 2.

Drug	Taken (µg/ml)	Found (µg/ml)	Recovery (%)	RSD (%)	Proposed method mean±SD	Refernce method mean±SD	t-test	F-test
Esomeprazole	5	4.96	99.36	1.93	99.48 ±0.48	99.72 ±0.5	0.89 5.15	0.25 2.31
	20	19.93	99.68	1.1				
	35	35.11	100.33	1.38				
	50	49.58	99.16	1.8				
	60	59.34	98.9	2.04				
Losartan K	20	20.01	100.05	0.20	99.89 ±0.08	99.3 ±1.46	0.93 -3.93	1.56 2.23
	40	40.00	100.00	0.13				
	60	59.99	99.97	0.07				
	80	79.98	99.96	0.08				
	82	81.9	99.84	0.04				
Paroxetine	20	19.8	99.95	0.85	100.3 ±0.59	99.84 ±0.87	1.04 4.38	0.65 2.26
	35	35	100.00	0.01				
	40	39.72	100.65	0.62				
	65	64.69	100.43	1.31				
	100	100.83	99.92	0.82				
Rabeprazole	30	31.80	105.1	1.77	100.13 ±0.08	100.6 ±0.90	1.92 4.38	2.22 2.26
	40	39.8	96.2	1.81				
	60	60.2	99.8	1.34				
	70	70	100	1				
	75	74.37	97.33	1.18				
Tamoxifen	25	25.25	101.2	1.42	99.92 ±0.51	99.6 ±0.21	1.51 8.91	0.71 2.35
	42	41.62	98.99	1.87				

	60	59.66	99.92	0.12				
	80	79.20	99.15	0.58				
	130	129	99.97	0.62				

Application of the proposed methods for the assay of Tablets Table 3.

Drug	Taken (µg/ml)	Found (µg/ml)	Recovery (%)	Proposed method mean±SD	Reference method mean±SD	t-test	F-test
Esomeprazole	30	30.32	100.62	100.04 ±1.5	99.15 ±0.66	1.67(5.02)	0.91(2.29)
	50	50.07	100.31				
	70	68.87	98.49				
Losartan K	10	9.79	98.74	99.4 ±0.76	99.64 ±0.87	1.64(3.96)	0.91(2.21)
	50	50.14	100.02				
	80	80.21	100.18				
Paroxetine	10	10	100.02	99.37 ±0.59	99.71 ±0.22	1.59(6.22)	1.41(2.34)
	20	19.78	98.72				
	30	29.91	99.92				
Rabeprazole	30	29.69	99.17	99.65 ±0.51	100.4 ±0.90	1.72(4.35)	0.986(2.26)
	60	59.89	99.90				
	70	70.01	100				
Tamoxifen	30	30.07	100.42	100.4 ±0.71	99.80 ±1.66	0.52(8.97)	0.04(2.35)
	60	60.12	100.16				
	70	69.99	99.99				

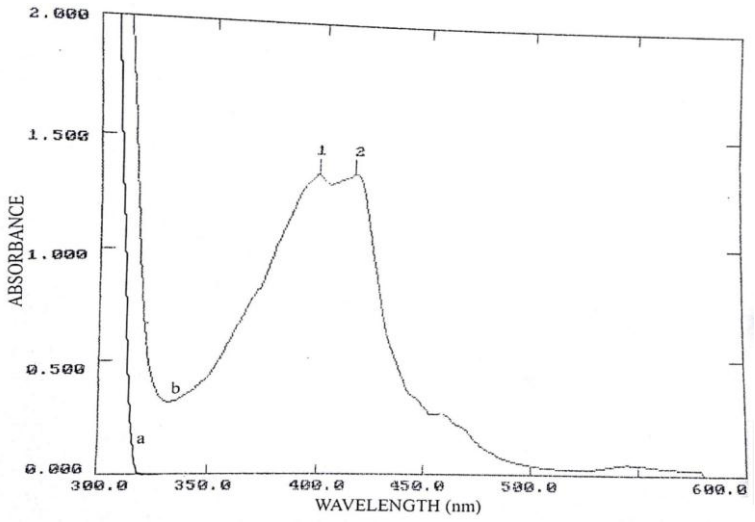


Fig. 6: Absorption spectra of a) Pure Drug b) TCNE in acetonitrile and c) its charge transfer complex with Rabeprazole

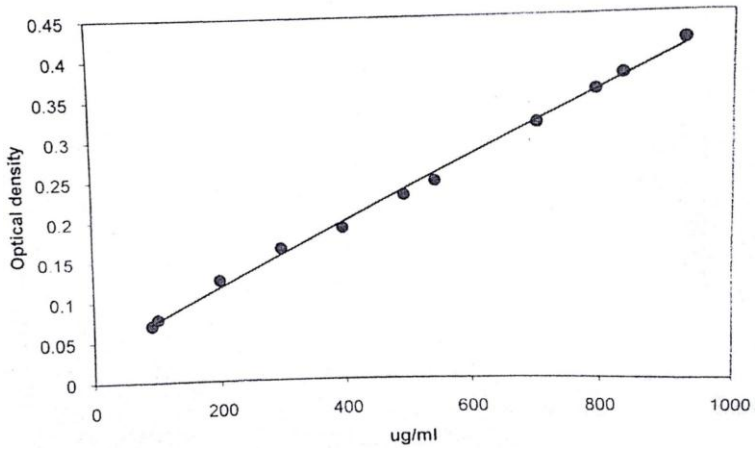


Fig. 7: Calibration curve for quantification of Rabeprazole using TCNE as analytical reagent.

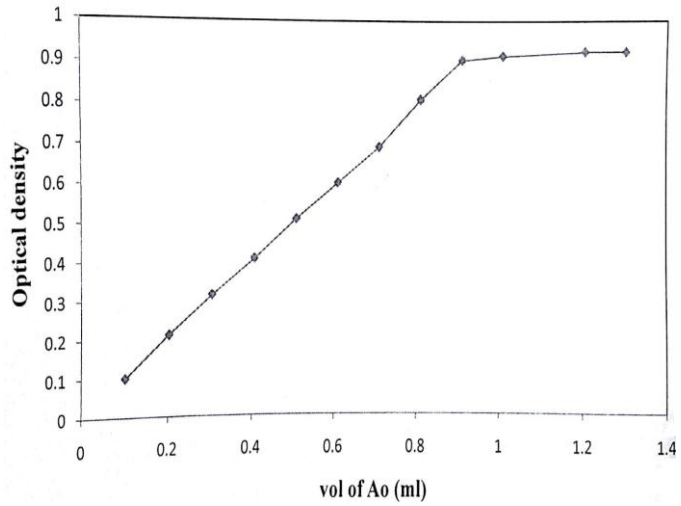


Fig. 8: Effect of volume of reagent on the optical density of the Ion-pair complex of TCNE and Esomeprozole.

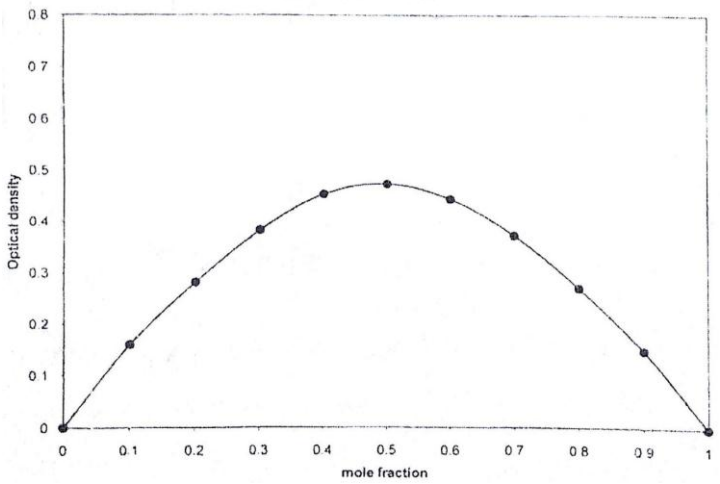


Fig.9 Job's continuous variation plot of TCNE and Esomeprozole.

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