SPECTROPHOTOMETRIC DETERMINATION OF DRUGS USING TETRACYANOETHYLENE AS ANALYTICAL REAGENT

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ABSTRACT

Five Drugs viz., Cloxacillin, Dextromethorphan, Famotidine, Pyrimethamine, Quetiapine were tested for the formation of charge transfer complexes with Tetracyanoethylene(TCNE). Each of these drugs turned the colourless reagent i.e TCNE., in CH₃CN, 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 10³ to 10⁴. 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: Spectrophotometer, TCNE, Cloxacillin, Dextromethorphan, Famotidine, Pyrimethamine Quetiapine.

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
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 Cloxacillin

Cloxacillin (Fig. 1) is a semisynthetic antibiotic in the same class as penicillin. It is sold under a number of trade names, including Cloxapen and Orbenin. It is used against staphylococci that produce beta-lactamase.

![Cloxacillin](image)

**Fig. 1: Cloxacillin**

\[(2S,5R,6R)-6-\{[3-(2-chlorophenyl)-5-methyl-oxazole-4-carbonyl]amino\}-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid\]

High performance liquid chromatography and reverse phase and high performance liquid chromatography are mainly used for the analysis of antibiotics by separation techniques\(^{[4-6]}\) and capillary electrophoresis\(^{[7]}\) is being increasingly employed due to its favourable characteristics (high efficiency, large flexibility, and low consumption of samples and reagent. Colorimetric determinations\(^{[8,9]}\), volumetric determinations\(^{[10]}\) and different spectroscopic techniques are used for the quantitative estimation of cloxacillin.\(^{[11-13]}\)

1.2 Dextromethorphan

Dextromethorphan (Fig. 2) is a non-narcotic morphinan derivative widely used as antitussive. It has attracted attention due to its anticonvulsant and neuroprotective properties. It is the most widely used nonopioid antitussive drug. It is considered to have a high abuse potential because of its psychological effects and ready accessibility. DXM is a powerful psychedelic and acts in four different “plateaus” where the nature of the response is dose dependant. In
the brain, it blocks the dopamine reuptake site, activates sigma receptor and blocks the open NMDA channel.

![Dextromethorphan (DXM)](image)

**Fig. 2: Dextromethorphan (DXM)**

\[((+)-3\text{-}methoxy\text{-}17\text{-}methyl\text{-} (9α,13α,14α)\text{-}morphinan)\]

The quantitative estimation of this drug has been a subject of considerable interest and was studied by many workers using different methods. Spectrophotometric determinations\cite{14,15}, techniques such as Liquid Chromatography and chemometric – assisted spectrophotometric methods\cite{16}, Gas Chromatography\cite{17,18}, High Pressure Liquid Chromatography\cite{19-24} and ion exchange separation\cite{25} have been used for the estimation of Dextromethorphan. Other methods reported for the analysis of dextromethorphan are capillary electrophoresis\cite{26}, and Hybrid Linear Analysis as a recent factor based multivariate calibration technique\cite{27}.

1.2 Famotidine

Famotidine (Fig. 3) is used in the treatment of duodenal ulcers, gastric ulcers, stress ulcers and gastritis.

![Famotidine](image)

**Fig. 3: Famotidine**

3-((2-(diaminomethyleneamino)thiazol-4-yl)methylthio)-N'-sulfamoylpropanimidamide.

Various methods have been reported for estimation of famotidine, which includes spectrophotometric methods\cite{28-30}, spectrophotometric and spectrofluorimetric method\cite{31}, and
flow injection analysis. Spectrophotometric method by means of N-bromosuccinamide and p- aminophenol[33] spectrophotometric determination via oxidation with cerium (IV)[34], iodometric determination[35], spectrophotometric determination using 2,6-dichlorophenol indophenol[36], titrimetric, spectrophotometric determination using chloramines-T[37] and chromatographic method[38] have been reported.

1.3 Pyrimethamine
Pyrimethamine (Fig. 4) is a medication used for protozoal infections. It is commonly used as an antimalarial drug (for both treatment and prevention), and is also used (combined with sulfadiazine) in the treatment of Toxoplasma gondii infections in immuno compromised patients, such as HIV-positive individuals. Pyrimethamine interferes with folic acid synthesis by inhibiting the enzyme dihydrofolate reductase. Folic acid is needed for DNA and RNA synthesis in many species, including protozoa.

![Pyrimethamine](image)

**Fig.4 Pyrimethamine**

5-(4-chlorophenyl)-6-ethyl- 2,4-pyrimidinediamine
Various methods used for the analysis of pyrimethamine are spectrophotometric determinations[39,40], physico-chemical studies[41], liquid chromatography/mass spectrophotometry[42], liquid chromatography[43] and derivative spectrophotometry.[44, 45]

1.4 Quetiapine
Quetiapine (Fig. 5) is an atypical antipsychotic drug with a unique receptor-binding profile belonging to a new chemical class, the dibenzothiazepine derivatives.[46–49] Quetiapine is an antagonist at a broad range of neurotransmitter receptors.[50] Quetiapine is used in the treatment of schizophrenia or manic episodes associated with bipolar disorder. These antipsychotics have a low incidence of extra pyramidal side effects and tardive dyskinesias compared to older antipsychotics. The advantages of the therapeutic profile of quetiapine
have led to increasing use in the clinical practice, which encourages the development of new pharmaceutical preparations. As a consequence, there is an increasing demand for new analytical methods for determination of pharmacokinetic parameters in bioequivalence studies. Some of these methods could be also employed in therapeutic drug monitoring. Due to inter-individual pharmacokinetic variability the dose has to be carefully titrated depending on the clinical response and tolerability of the individual patient.

![Fig. 5 Quetiapine](image)

2-(2-(4-dibenzo[b,f][1,4]thiazepine- 11-yl- 1-piperazinyl)ethoxy)ethanol
Several HPLC methods for the determination of Quetiapine have been reported. Some gas chromatography–mass spectrometry (GC–MS) methods have also been employed, however here Quetiapine needs to be derivatized before analysis.

The proposed mechanism Scheme-1
Donor-acceptor complex or Charge transfer complex formation

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 chloro benzene and vacuum sublimed to get pure white crystals of TCNE. A stock solution of 100mg/100ml (w/v) \(7.812 \times 10^{-3} \text{M}\) in acetonitrile 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\(_4\)OH 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 aceetonytrile, AR grade methanol, either NaOH and NH\(_4\)OH all of them are supplied by SD fine Chemicals, Mumbai.

3 EXTRATIONS OF DRUGS FOR PHARMACEUTICAL ANALYSIS

3.1 Cloxacillin

Four capsules (Klox-250mg) were weighed and the average weight was determined and these were powdered. The powder capsules equivalent to 50mg of cloxacillin is taken in a 100ml volumetric flask. Then the drug was dissolved using 100ml methanol and shaken well. The content was filtered in a beaker using Whatmann filter paper. A few millilitres of solvent is added to wash the residue. Methanol was evaporated and acetonitrile was added and heated on water bath for the complete dissolution of drug and serial dilutions are done for the analysis.

3.2 Dextromethorphan

The contents of ten tablets (Lastuss-CT-10mg) were crushed, powdered, weighed out and the average weight of tablets were determined. An accurately weighed powdered tablets equivalent to 50mg of dextromethorphan was dissolved in 20ml double distilled water with shaking for 5minutes and then filtered. The dextromethorphan solution was then taken in a
separating funnel containing ether followed by the addition of 0.1N sodium hydroxide. The content of separating funnel were mixed well and shaken for 5 minutes. The two layers are separated. Ethereal layer is evaporated and the residue is dissolved in acetonitrile.

3.3 Famotidine
Twenty tablets (Autidine - 20mg) 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 5 minutes. The mixture was mixed well, filtered and evaporated to dryness. Residue was dissolved in acetonitrile heating on waterbath 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.

3.4. Pyrimethamine
Five tablets (Daramin- 25mg) were weighed accurately and ground to a fine powder. An amount of powder equivalent to 50mg of pyrimethamine was weighed into a 100ml volumetric flask, 100ml of methanol was added and shaken thoroughly for about 10 minutes and filtered using a quantitative filter paper in a beaker. The residue was washed with methanol for complete recovery of the drug. Methanol was evaporated by heating on water bath and then to the content acetonitrile is added and heated on water bath for the complete dissolution of the drug. The solution is diluted for the analysis.

3.5. Quetiapine fumerate
For the analysis of pharmaceutical formulations 10 tablets (Q-Pin – 25mg) were weighed and pulverised. A weighed quantity of the powdered tablets equivalent to 50mg of quetiapine fumerate was transferred into a volumetric flask containing methanol. The content was filtered through Whatmann filter paper into a beaker. The content was washed with a few ml of methanol and washings were passed into the beaker. Methanol was then evaporated by heating on water bath and acetonitrile is added. From these solution aliquots volumes covering the working concentration range were transferred into 10ml volumetric flask and were determined.

4. SPECTRA
The spectra of ion-pair Charge transfer complexes were record in CH$_3$CN 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 was noted 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, Cloxacillin, Dextromethorphan, Famotidine, Pyrimethamine, and Quetiapine showed color changes when mixed with TCNE in CH$_3$CN. 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 acetonitrile. 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 20µg/ml of Dextromethorphan, 60µg/ml of Cloxacillin, 35µg/ml of
Quetiapine, 35µg/ml of Famotidine, 40µg/ml of Pyrimethamine. It was found that 0.8 ml of 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°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 harmonisation (ICH) viz, selectivity, sensitivity, precision, accuracy, linearity LOD < LOQ Sandell’s sensitivity and robusteness. 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 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 “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 as ample that can be detected, but not necessarily quantitated as an exact value” and Limit of Quantification “The Lowest amount of analyte in as ample 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 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.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, \(\varepsilon\).

\[
\frac{A_0}{d}=1/K (D_o)\varepsilon+1/ \varepsilon
\]

Above equation is known as BH equation and a plot of \(A_0/d\) Vs \(1/D_o\) is a straight line from whose slope and intercept the K and \(\varepsilon\) are determined. The BH method however demands the concentration of donor \(D_o >> A_o\) (\(D_o\) should be 20 to 100 times acceptor concentration) and many times the correct separation of K and \(\varepsilon\) is also doubtful. Many works used the Benesi – Hildebrand method without fulfilling the condition \(D_o >> A_o\) and the values of \(\varepsilon\) obtained varied widely. The \(\varepsilon\) reported for TCNE: are \(9 \times 10^3\) to \(4.17 \times 10^4\) \text{lit mole}^{-1} \text{cm}^{-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 (2.97×10^{-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.

\[ K = \frac{(d/ \varepsilon)}{[Ao-(d/ \varepsilon)][Do-(d/ \varepsilon)]} \]

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

The stiochiometry 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 TCNE with drug Dextromethorphan is presented in fig.9.

**Structure activity relation**

From the slopes of calibration it is clear that the donor abilities of the drug are in the order: Pyromethamine > Famotidine > Dextromethorphen > Quetiapine > Cloxacillin.

From the structures of Drugs the order of the basicity is Dextromethorphan and Quetapinine are greater than Famtidine, Paramythamine greater than Cloxacilline.

**10. APPLICATIONS OF THE PROPOSSED 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.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Cloxacillin</th>
<th>Dextromethorphan</th>
<th>Famotidine</th>
<th>Pyrimethamine</th>
<th>Quetiapine</th>
</tr>
</thead>
<tbody>
<tr>
<td>(\lambda_{\text{max}}) (nm)</td>
<td>420</td>
<td>420</td>
<td>420</td>
<td>420</td>
<td>420</td>
</tr>
<tr>
<td>Beer’s law limits ((\mu)g ml(^{-1}))</td>
<td>Mar-63</td>
<td>Feb-19</td>
<td>Apr-32</td>
<td>May-40</td>
<td>Apr-32</td>
</tr>
<tr>
<td>Molar absorptive (L mol(^{-1}) cm(^{-1}))</td>
<td>8790</td>
<td>14940</td>
<td>7890</td>
<td>6990</td>
<td>8640</td>
</tr>
<tr>
<td>Formation constant, K, M(^{-1})</td>
<td>550 ± 10</td>
<td>1370 ± 50</td>
<td>390 ± 10</td>
<td>410 ± 10</td>
<td>510 ± 10</td>
</tr>
<tr>
<td>Sandal sensitivity ((\mu)g cm(^{-1}))</td>
<td>0.0571</td>
<td>0.0234</td>
<td>0.0567</td>
<td>0.0574</td>
<td>0.0469</td>
</tr>
<tr>
<td>Slope b</td>
<td>0.0175</td>
<td>0.0426</td>
<td>0.0176</td>
<td>0.0174</td>
<td>0.0213</td>
</tr>
<tr>
<td>Intercept (a)</td>
<td>0.0078</td>
<td>0.01095</td>
<td>0.00168</td>
<td>-0.0086</td>
<td>0.017</td>
</tr>
<tr>
<td>Correlation coefficient</td>
<td>0.9996</td>
<td>0.9983</td>
<td>0.998</td>
<td>0.9997</td>
<td>0.9971</td>
</tr>
<tr>
<td>Standard deviation of intercepts (%n=5)</td>
<td>0.0025</td>
<td>0.0031</td>
<td>0.00327</td>
<td>0.0072</td>
<td>0.00525</td>
</tr>
<tr>
<td>Limit of detection ((\mu)g ml(^{-1}))</td>
<td>0.4662</td>
<td>0.2396</td>
<td>0.6121</td>
<td>1.3656</td>
<td>0.8138</td>
</tr>
<tr>
<td>Limit of quantification ((\mu)g ml(^{-1}))</td>
<td>1.3988</td>
<td>0.7187</td>
<td>1.8362</td>
<td>4.0969</td>
<td>2.4416</td>
</tr>
<tr>
<td>Regression equation Y=bx+a</td>
<td>Y=0.0078+0.0175x</td>
<td>Y=0.010+0.0426x</td>
<td>Y=0.00168+0.0176x</td>
<td>Y=0.0078+0.0175x</td>
<td>Y=0.00168+0.0176x</td>
</tr>
<tr>
<td></td>
<td>x is Conc ((\mu)g ml(^{-1}))</td>
<td>x is Conc ((\mu)g ml(^{-1}))</td>
<td>x is Conc ((\mu)g ml(^{-1}))</td>
<td>x is Conc ((\mu)g ml(^{-1}))</td>
<td>x is Conc ((\mu)g ml(^{-1}))</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Drug</th>
<th>Taken((\mu)g/ml)</th>
<th>Found((\mu)g/ml)</th>
<th>Recovery (%)</th>
<th>RSD (%)</th>
<th>Proposed method mean±SD</th>
<th>Referene method mean±SD</th>
<th>t-test</th>
<th>F-test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cloxacillin</td>
<td>20</td>
<td>19.91</td>
<td>99.58</td>
<td>1.77</td>
<td>99.95±0.94</td>
<td>98.6±0.58</td>
<td>2.04</td>
<td>0.75-2.45</td>
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<tr>
<td></td>
<td>30</td>
<td>29.62</td>
<td>98.73</td>
<td>1.55</td>
<td></td>
<td></td>
<td>-9.01</td>
<td></td>
</tr>
<tr>
<td></td>
<td>40</td>
<td>40.41</td>
<td>101.04</td>
<td>1.31</td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>50</td>
<td>49.19</td>
<td>98.39</td>
<td>1.69</td>
<td></td>
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<td></td>
<td>60</td>
<td>58.2</td>
<td>97</td>
<td>2.06</td>
<td></td>
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<td>Dextromethorphan HBr</td>
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<td>20.01</td>
<td>100.07</td>
<td>0.22</td>
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<td>82</td>
<td>81.9</td>
<td>99.88</td>
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<tr>
<td>Famotidine</td>
<td>20</td>
<td>19.8</td>
<td>100.52</td>
<td>0.89</td>
<td>100.3±0.59</td>
<td>99.84±0.87</td>
<td>1.04</td>
<td>0.65-2.26</td>
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<tr>
<td></td>
<td>35</td>
<td>35</td>
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<td>40</td>
<td>39.72</td>
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<td>0.67</td>
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<td></td>
<td>65</td>
<td>64.69</td>
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<td>1.34</td>
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</tbody>
</table>
### Application of the proposed methods for the assay of Tablets Table 3.

<table>
<thead>
<tr>
<th>Drug</th>
<th>Taken (µg/ml)</th>
<th>Found (µg/ml)</th>
<th>Recovery (%)</th>
<th>Proposed method mean±SD</th>
<th>Reference method mean±SD</th>
<th>t-test</th>
<th>F-test</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>100.83</td>
<td>99.96</td>
<td>0.86</td>
<td>100.13 ±0.08</td>
<td>100.6 ±0.90</td>
<td>1.92</td>
</tr>
<tr>
<td>Pyrimethamine</td>
<td>30</td>
<td>32.13</td>
<td>107.1</td>
<td>2.07</td>
<td>100.6 ±0.90</td>
<td>1.92</td>
<td>4.38</td>
</tr>
<tr>
<td></td>
<td>40</td>
<td>39.8</td>
<td>96.2</td>
<td>1.81</td>
<td>100.6 ±0.90</td>
<td>1.92</td>
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**Fig. 6 Absorption Spectra of a) pure drug c) it's Charge transfer complex with Dextromethorphan**
Fig. 7 Calibration curve for quantification of Dextromethorphan using TCNE as analytical reagent.

Fig. 8 Effect of volume of reagent on the optical density of the Io-pair complex of TCNE and Pyrimethamine.

Fig. 9 Job’s Continuous variation plo of TCNE and Pyrimethamine.
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