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QUANTITATIVE DETERMINATION OF DRUGS IN BULK AND PHARMACEUTICAL DOSAGE FORMS BY USING 2,3-DICHLORO-5,6-DICYANOBENZOQUINONE

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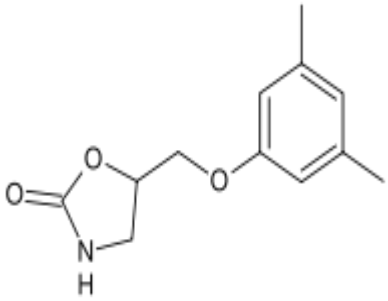
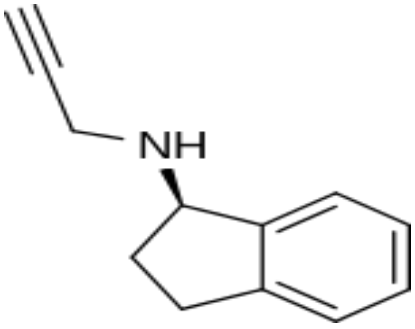
ABSTRACT

The present work narrates determination of drugs *viz*,. Metaxalone (MTX), Rasagiline (RSG), Aprepitant (APR) and Linezolid (LZD) based on formation of Charge Transfer Complex of drugs as n-electron donor with 2,3-dichloro-5,6-dicyanobenzoquinone(DDQ) as π-acceptor in bulk and pharmaceutical dosage forms. The selected drugs turned the yellow color solution of DDQ in Acetonitrile to thick brown color and exhibited a band at 585nm due to the formation of Complex of drugs with DDQ. Under the optimized experimental conditions, Beer' s law is obeyed over the concentration ranges of 15-75 µg/ml, 10-50 µg/ml, 5-25 µg/ml and 10-50 µg/ml for, MTX, RSG, APR and LZD respectively. The sensitivity, accuracy of methods, effect of reagent concentrations, polarity of solvents and effect of reaction time have been studied and optimized. The stoichiometric relationship was determined by Job's continuous method and is found to be 1:1 in each case. 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, 2,3-dichloro-5,6-dicyanobenzoquinone,Charge-transfer complex, Quantification and Validation.



Metaxalone



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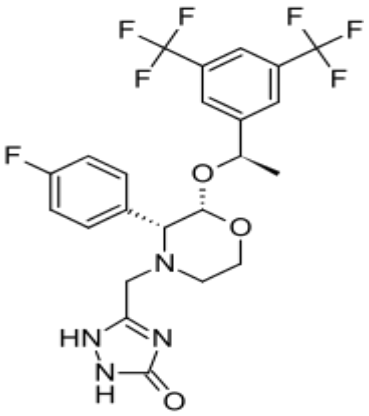
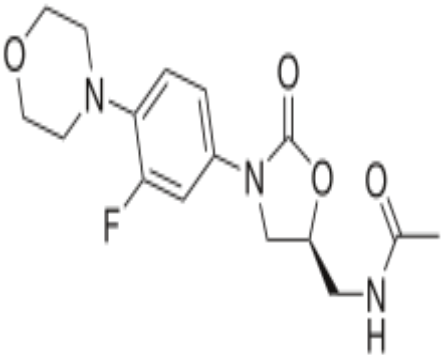
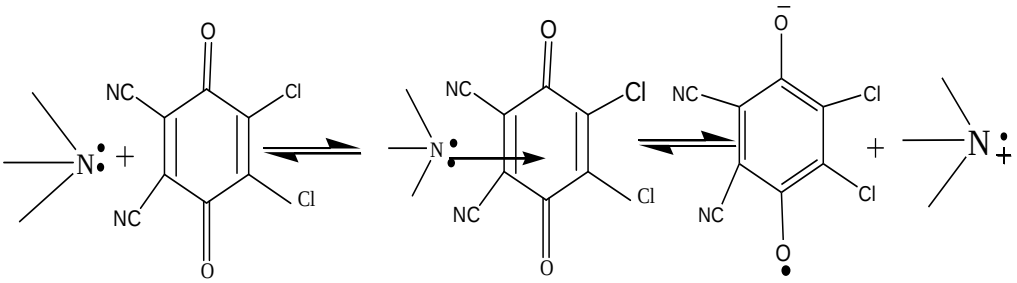


Linezolid



Aprepitant



Donor (Drug) Acceptor (DDQ) Charge transfer complex anion cation

INTRODUCTION

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.[1] Metaxalone belongs to non benzodiazepine antispasmodics with a structure similar to mephenaxalone nucleus. It acts through inhibiting inter neuronal 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.[2]

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), and UV spectroscopy, gas chromatography with flame ionization detection and gas chromatography with mass detection.[3]

Rasagiline

Rasagiline mesylate is chemically named as (1R) -2,3-dihydro-N-2-propynyl-1H-inden-1-amine.[4] Rasagiline is a very effective, selective and irreversible second generation monoaminoxidase inhibitor with the selectivity for type B enzyme (MAO–B). The useful effects seen in dopaminergic motor disfunction models of rasagiline should be sourced of high dopamine surface and then increased dopaminergic activity. 1-Aminoindane is an active main metabolite and is not a MAO–B inhibitor. Rasagiline is indicated in treatment of idiopathic Parkinson disease as monotherapy (without concomitant levodopa treatment) and as adjuvant treatment (with concomitant Levodopa treatment) of patients having dosage end waving.[5]

Through survey of literature reveals that a few analytical methods have been reported for estimation of Rasagiline mesylate *viz.*, determination of rasagiline mesylate in human plasma and its pharmacokinetics by LC–MS–MS , ion chromatography, reversed phase HPLC methods, by using UV spectrophotometric methods, thermodynamic dissociation constants of rasagiline by spectrophotometric pH titration data.[6]

Aprepitant

Aprepitant is chemically named as 5-([(2R,3S)-2-((R)-1-[3,5-bis (trifluoromethyl) phenyl] ethoxy) 3-(4-fluorophenyl) morpholino] methyl)-1H-1,2,4-triazol-3(2H)-one.[7] Aprepitant is a selective high affinity antagonist of human substance.

P/neurokini 1(NK1) receptors and it has little or no affinity for serotonin (5-HT3), dopamine, and corticosteroid receptors. A largenumber of drugs are available for prevention of PONV.[1] of which 5-HT3 receptor antagonists have occupied an important position because of their better efficacy and side effect profile with a disadvantage that it prevents only acute emesis.

A newer class of drugs namely neurokinin receptor antagonists provides an additional advantage of preventing both acute and delayed emesis.[8]

Literature survey reveals that the drug can be estimated by RP-HPLC in Capsules, Stability indicating RP-HPLC, rapid liquid chromatography-tandem mass spectrometry method, quantification of process related impurities by RP-LC method and UPLC methods.[9] Complementarily of UV-PLS and HPLC for the simultaneous evaluation of antiemetic drugs.[10]

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 *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 paper reports simple, direct, and sensitive spectrophotometric methods for determination of drugs by using DDQ 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 DDQ solution of 1mg/ml (4.4×10^{-3}M) 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 ml of DDQ solution in acetonitrile was added and remaining volume was made up by solvent. The absorbance of thick brown colored solution was recorded after 15min of mixing against reagent blank at 585nm is plotted against the corresponding concentrations (µg/ml) of the drug to construct the calibration curve.

DETERMINATION OF DRUGS IN DOSAGE FORM

Metaxalone

Ten Tablets were weighed, finely powdered and an accurately weighed sample of powdered tablets equivalent to 50 mg of metaxalone was transferred into 25 mL volumetric flask and dissolved in about 25ml of methanol. The contents of the flask were swirled, sonicated for 20 minutes. The mixture was filtered with Whatmann filter paper 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.

Aprepitent

Ten Tablets were weighed, finely powdered and an accurately weighed sample of powdered tablets equivalent to 25mg of Aprepitent into 25 ml volumetric flask and dissolved in about 25ml of methanol. The contents of the flask were swirled, sonicated for 15 minutes. The mixture was filtered with Whatman filter paper 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.

Rasagiline

Ten tablets were accurately weighed and finely powdered. A quantity of the powdered tablets equivalent to 50mg of rasagiline was transferred to a 50 mL volumetric flask and dissolved in

about 50ml of methanol. The contents of the flask were swirled, sonicated for 20 minutes. The mixture was filtered with Whatmann filter paper 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.

Linezolid

Ten tablets were accurately weighed and finely powdered. A quantity of the powdered tablets equivalent to 50mg of rasagiline was transferred to a 50 mL volumetric flask and dissolved in about 50ml of methanol. The contents of the flask were swirled, sonicated for 20 minutes. The mixture was filtered with Whatmann filter paper 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.

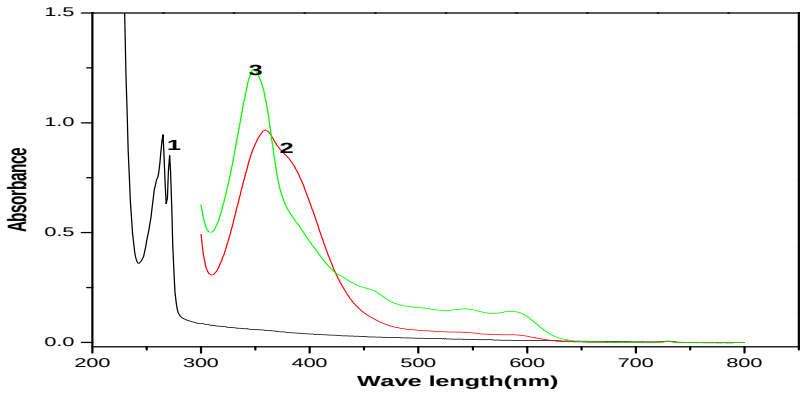


Fig (1) :(1) Rasagiline in Acetonitrile, (2) DDQ in Acetonitrile and (3) Charge transfer complex ofRasagiline with DDQ.

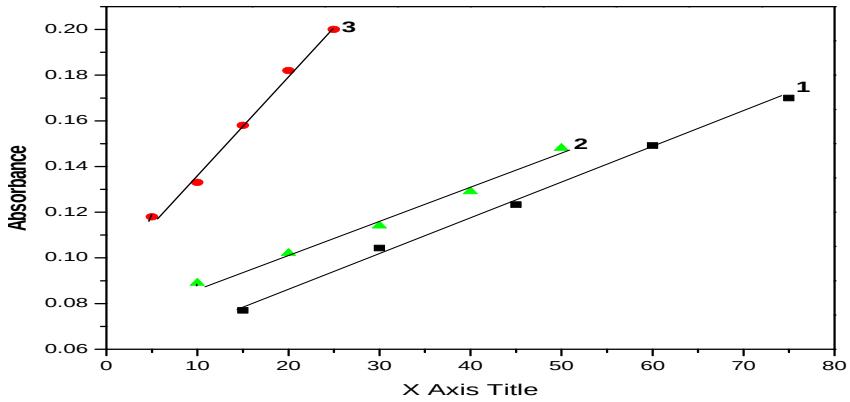


Fig (2): Calibration curves of DDQ with (1) Metaxalone, (2) Rasagiline and (3) Aprepitant.

EFFECT OF CONCENTRATION OF ACCEPTOR

To establish the optimum concentration of reagent ,Metaxalone 75 µg/ml, Rasagiline 50 µg/ml ,Aprepitant 25 µg/ml and Linezolid 50 µg/ml were react with different volumes of DDQ (4.4×10^{-3}M) .The results showed that the highest absorbance was obtained with 1ml .Hence 1ml of reagent was used for the determination of drugs.

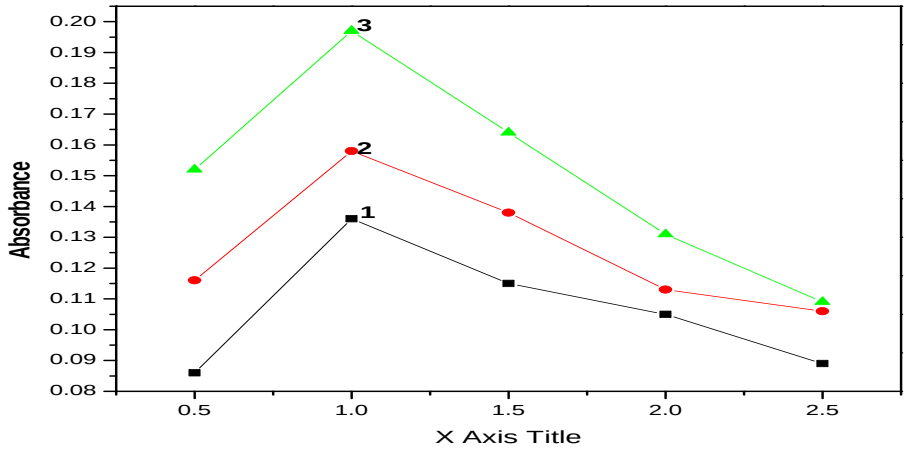


Fig (3): Effect of volume of reagent on the optical density of the Ion - pair complex of DDQ and (1) Rasagiline, (2) Aprepitant and (3) Linezolid.

EFFECT OF SOLVENT

Both polar and non-polar solvents such as methanol, acetone, chloroform, 1, 2-dichloroethane and acetonitrile were used to select elegant solvent for the analysis of drug.Acetonitrile is found to be suitable solvent for DDQ 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 DDQ with drugs resulted in the formation of ion- pair complexes which stabilized with in 15 min of mixing. The developed color remained stable at room temperature for about an hour. After a day all solutions are decolorized.

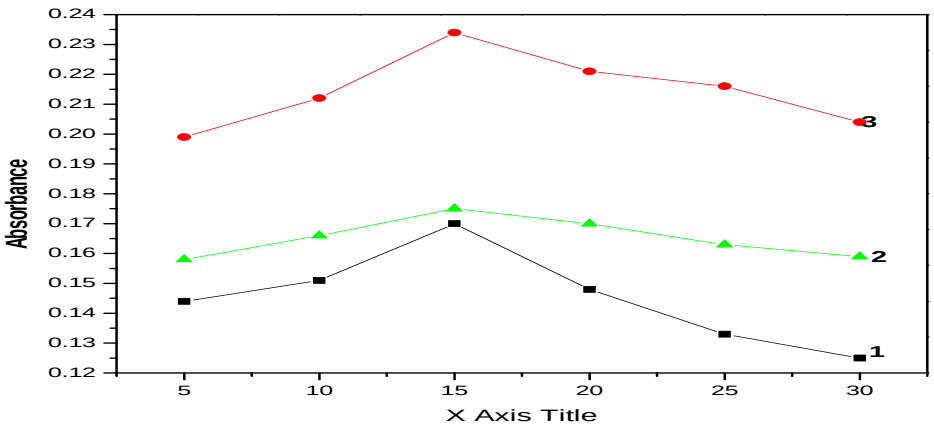


Fig (4): Effect of reaction time on formation of charge transfer complexes of DDQ and (1) Rasagiline, (2) Aprepitant and (3) Linezolid.

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 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. 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.

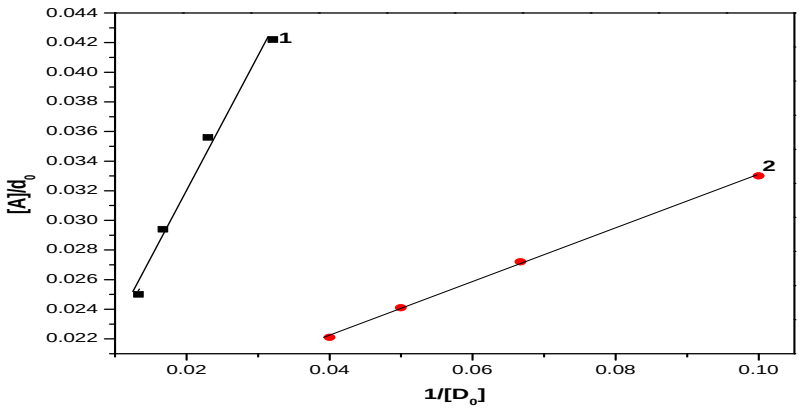


Fig (5): Benesi – Hildebrand plot of DDQ with (1) Metaxalone and (2) Aprepitant.

STOICHIOMETRY

The stoichiometry of each of the complex has been determined from Job's continuous variation method and found to be 1:1 in each case. A typical Job's plot of selected drugs with DDQ is presented in (Fig-6).

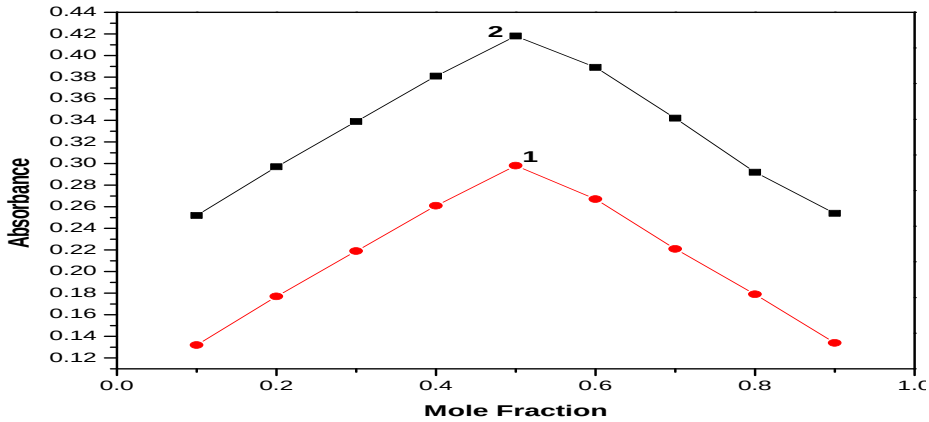


Fig (6): Job`s continuous variation plot of DDQ with (1) Aprepitant and (2) Rasagiline.

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

Drugs name parameters	Metaxalone	Rasagiline	Aprepitant	Linezolid
λ max, nm	585	585	585	585
Beer's law limit (µg/ml)	15-75	10-50	5-25	10-50
Molar absorptivity ($L\ mol^{-1}\ cm^{-1}$)	2205	8900	13300	3860

Slope(specific absorptivity),b	0.0014	0.0012	0.0043	0.0012
Intercept, a	0.0625	0.0778	0.0919	0.1492
Correlation coefficient, r	0.9988	0.9983	0.9986	0.9997
Sandell's sensitivity (μgcm^{-2})	0.7142	0.8334	0.2325	0. 8333
Formation constant, K(M^{-1})	446	555	625	490
Standard deviation of intercepts (n=6)	0.0019	0.0015	0.0035	0.0022
Limit of detection (µg/ml)	4.4785	4.125	2.6805	6.05
Limit of quantification (µg/ml)	13.5714	12.5	8.1395	18.3334
Regression equation y=a+bx; x=concentration of drug(µg/ml)	0.0625 + 0.0014x	0.0778 + 0.0012x	0.0919 + 0.0043x	0.1492 + 0.0012x

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

	Metaxalone	**Rasagiline**	**Aprepitant**	**Linezolid**
Amount taken (µg/ml)	15 30 45 60	10 20 30 40	5 10 15 20	10 20 30 40
Amount Found (µg/ml)	14.99 29.99 45.04 60.02	9.99 20.01 29.98 39.99	4.99 10.03 14.98 20.02	9.98 19.99 29.98 40.02
% Recovery	99.93 99.96 100.08 100.03	99.9 100.05 99.93 99.97	99.89 100.03 99.98 100.01	99.8 99.95 99.93 100.05
% RSD	0.04 0.03 0.02 0.04	0.06 0.03 0.04 0.01	0.11 0.01 0.02 0.03	0.05 0.04 0.09 0.03
Proposed Mean ± SD	100.0 ± 0.067	99.96 ± 0.065	99.97 ± 0.058	99.93 ± 0.102
Ref Mean ± SD	99.4 ± 0.102	100.0 ± 1.43	99.99 ± 0.03	100.07 ± 1.63
t-test	0.7 (2.44)	2.3333 (2.57)		2.2908 (2.44)

F-test	0.4327 (3.05)	0.0021 (3.40)	3.7333 (3.05)	0.0039 (3.05)

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

	Metaxalone	**Rasagiline**	**Aprepitant**	**Linezolid**
Amount taken (µg/ml)	30 45 60 75	20 30 40 50	10 15 20 25	20 30 40 50
Amount Found (µg/ml)	29.99 45.01 59.99 75.03	19.99 30.05 39.98 50.02	10.03 14.99 20.01 24.99	20.01 29.99 40.05 50.03
% Recovery	99.97 100.02 99.98 100.04	99.95 100.16 99.95 100.04	100.3 99.93 100.05 99.96	100.05 99.96 100.12 100.06
% RSD	0.047 0.102 0.076 0.044	0.034 0.026 0.039 0.046	0.034 0.035 0.012 0.018	0.033 0.046 0.035 0.017
Proposed Mean ± SD	100.002 ± 0.03	100.02 ± 0.09	100.06 ± 0.04	100.04 ± 0.15
Ref Mean ± SD	100.14 ± 0.11	102.1 ± 1.66	100.01 ± 0.03	99.98 ± 0.77
t-test	1.64 (2.57)	0.89 (2.44)		1.71 (2.44)
F-test	0.09 (3.40)	0.01 (3.05)	1.77 (3.05)	0.03 (3.40)

CONCLUSION

DDQ forms charge transfer complexes with selected drugs and exhibits band at 585nm.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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