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Development, validation and stoichiometric studies of spectrophotometric methods for determination of six antipsychotic drugs in bulk, spiked human plasma and pharmaceutical formulations based on charge transfer complexation

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ABSTRACT

A spectrophotometric method was developed for the determination of six antipsychotic drugs, namely, Ethosuximide (I), Amisulpride (II), Flupentixol (III), Citalopram (IV), Buspirone (V) and Fluoxetine (VI) through charge transfer (CT) complex formation with 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ), 2,5-dichloro-3,6-dihydroxy-1,4-benzoquinone (p-chloranilic acid, CL) and 7,7,8,8-tetracyanoquinodimethane (TCNQ). These π acceptor systemswere found to react with these drugs to produce stable complexes. The formation of such complexes with CL were synthesized and characterized by elemental analysis,FT-IR and UV–VIS measurements. The different experimental parameters that affect the spectrophotometric intensity were carefully studied, at the optimum reaction condition the rectilinear calibration graphs were obtained in the concentration range 0.0005 -50 µg/ml for the investigated drugs.The limits of detection ranged from 0.0002 to 0.016µg/ml. The proposed procedures could be applied successfully for the determination of the investigated drugs in their pharmaceutical dosage forms with a good precision and accuracy compared to official and reported methods. Also they were applied to determine spiked human plasma samples. The Stoichiometry of the CT complexes of DDQ, TCNQ and p-CL with the proposed drugs determined by Job's method and the stability constants (K_{ct}) for the reported CT complexes were calculated according to the Benesi–Hildebrand equation.

Keywords: Spectrophotometric, Anti schizophrenic drugs, Charge-transfer complexes (CT), DDQ, TCNQ, p-CL, Job's method, Benesi–Hildebrand, elemental analysis, FTIR and UV–VIS.

INTRODUCTION

Antipsychotic drugs are the primary intervention for stabilization of acute psychotic episodes and prevention of recurrences. They primarily used to manage psychosis such as (delusions, hallucinations, disordered thought, schizophrenia, schizoaffective disorder, bipolar disorder, psychotic depression, dementia and insomnia). They are frequently encountered in emergency toxicology screening, drug-abuse testing and forensic medical examinations [1]. In this study, six antipsychotic drugs namely; Ethosuximide (I), Amisulpride (II), Flupentixol (III), Citalopram (IV), Buspirone (V) and Fluoxetine (VI) were studied. Their structures are shown in Figure [1].

Ethosuximide is mainly used as anticonvulsant, while Both Amisulpride and Flupentixol are used for treatment of schizophrenia, mania and bipolar disorder.Citalopram, Fluoxetine are antidepressant and Buspirone is used mainly for treatment of anxiety disorders [2].

Various chromatographic methods are used for the determination of these drugs and their metabolites in biological fluids such as liquid chromatography couples with mass spectroscopy [LC/MS] for determination of Ethosuximide in human plasma [3]. Also liquid chromatography tandem mass spectroscopy (LC/MS/MS) method is reported for determination of Amisulpride, Flupentixol and Fluoxetine[4-7].

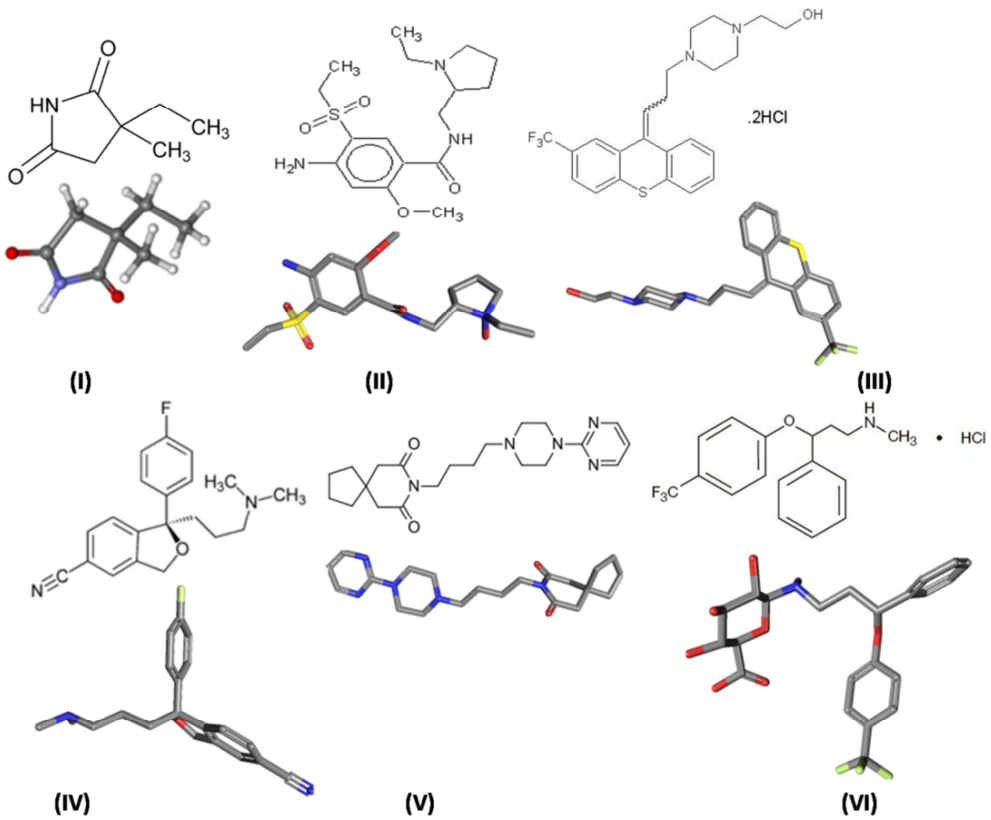

Figure 1: The 1-D and 3D structure Ethosuximide (I), Amisulpride (II), Flupentixol (III), Citalopram (IV), Buspirone (V) and Fluoxetine (VI)

Ultra-performance liquid chromatography (UPLC) coupled with tandem mass spectrometry [8-9] and high performance liquid chromatography (HPLC) [10-15] are new applicable methods used for routine therapeutic drug detection and monitoring including antiepileptic drugs. HPLC coupled with fluorescence [16-17], UV [18-19] and chemiluminescence [20] detection methods are used for determination of II, III, IV and V respectively in human plasma and urine. Liquid chromatography-Elctrospray ionization tandem mass LC-ESI-MS/MS method is reported to be used for quantitation of Fluoxetine [21] and enantioselective extraction of (+)-(S)-Citalopram and its main metabolites [22].

Fluoxetine hydrochloride is determined in capsules by TLC-spectrodensitometry[23]. Another reported Simple and rapid chromatographic methods are Gas chromatography (GC) [24-28], GC/MS [29-31] and GC/MS MS [32] which are used for Simultaneous quantitative determination of Ethosuximide, Amisulpride, Flupentixol and Citalopram.

Several electrophoretic methods are reported for determination of Amisulpride and Citalopram [33-36]while adsorptive square wave voltammetry is used for determination of Fluoxetine and Citalopram [37].

As the methods used for determination of the above drugs are expensive and time consuming, the main aim is to develop fast, simple, inexpensive methods that can readily be adapted for routine analysis at relatively low cost.
Charge transfer complex forming reactions have been used in the determination of electron-donating basic compounds through the interaction with π-acceptors such as 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ), dichloro-3,6-dihydroxy-1,4-benzoquinone (p-CL) and 7,7,8,8-tetracyanoquinodimethane (TCNQ) [16-18]. Our

investigated drugs contain tertiary amino group in their molecular structure, it represents a basic centre with the availability of non-bonding electron as donors.

The described facts encouraged attempts to use the formation of a complex between π-acceptors and our proposed drugs for the determination of them and their dosage forms. At the same time, the spectroscopic features, such as the association constant, and the molar ratio of reaction were determined. The results obtained by the above spectrophotometric method were compared statistically with a simple reported reversed- phase HPLC procedure.

EXPERIMENTAL SECTION

1. Chemicals

I. Amisulpride, Buspirone and Fluoxetine working standards were provided by Sigma Pharmaceutical Industries Company while Ethosuximide, Flupentixol and Citalopram working standards were obtained from Deltapharm Company

II. Plasma samples were purchased from the central blood bank of Tanta University Hospital. Copper chloride, DDQ, CL and TCNQ were prepared fresh daily. All reagents used were of analytical grade.

III. DDQ, p-CL and TCNQ (Sigma Chemical Co., USA) were prepared as1×10^{-3}mol l^{-1} in Acetonitrile. Aliquots of these solutions were diluted with the same solvent to obtain solutions containing appropriate concentrations to obtain optimal spectrophotometric absorbance for each drug.

2. Instrumentation

The elemental analysis of the carbon, hydrogen and nitrogen contents were performed using Carlo Erba instruments EA 1110. The electronic absorption spectra of Acetonitrile solutions of the donor, acceptors and resulting CT complexes were recorded over a wavelength range of 200-900 nm using Shimadzu U.V-160A spectrophotometer-double beam. The instrument was equipped with a quartz cell with a 1.0 cm path length. The mid-infrared (IR) spectra (KBr discs) within the range of 5000-400 cm^{-1} for the solid CT complexes were recorded on a Shimadzu FT-IR spectrophotometer.

3. Preparation of standard stock solutions and spiked human plasma samples

Stock solutions for Ethosuximide (I), Amisulpride (II), Flupentixol (III), Citalopram (IV), Buspirone (V) and Fluoxetine (VI) were prepared in Acetonitrile to contain 1 mg/ml.

Serial standard solutions were prepared in the same solvent having concentrations ranging from 10 to 50 µg/ml, 10 to 35 µg/ml, 0.5 to 10 ng/ml, 0.1 to 4 µg/ml, 0.3 to 7 µg/ml and 0.05 to 0.55 µg/ml of I, II, III, IV, V and VI respectively.

Serial standard solutions were spiked in human plasma and vortex mixed. Spiked human plasma samples were mixed with methanol and centrifuged for 15 minutes to separate the precipitated protein. The clear supernatant was filtered to obtain solutions in concentrations ranging from 12 to 45 µg/ml, 13 to 30 µg/ml, 0.8 to 8ng/ml, 0.5 to 3.7 µg/ml, 0.7 to 6.3 µg/ml and 0.08 to 0.5 µg/ml of I,II, III, IV, V and VI respectively.

4. Preparation of synthetic mixtures

Synthetic mixtures containing drugs along with various excipients, additives and other non active ingredients commonly used in pharmaceutical formulations were prepared.

Two synthetic mixtures containing Ethosuximide were prepared. The first mixture contained 250 mg Ethosuximide, polyethylene glycol 400, D&C yellow No. 10, FD&C red No. 3, gelatin, glycerin and sorbitol. The second mixture contained 250 mg Ethosuximide, citric acid anhydrous, FD&C red No. 40, FD&C yellow No. 6, flavor, glycerin, purified water, saccharin sodium, sodium benzoate, sodium citrate and sucrose.

A synthetic mixture containing (400 mg) of Amisulpride, methylcellulose (42.6 mg), sodium starch glycollate (0.5 gm), magnesium stearate (0.08 gm), Eudragit E100 (0.2 gm), purified talc (33.6 mg), macrogol 6000 (50.5 mg), titanium dioxide (52.9 mg), lactose and microcrystalline cellulose to 3.74 gm was prepared.

Flupentixol were prepared in a two synthetic mixtures. The first mixture contained 0.5 mg Flupentixol, lactose monohydrate, maize starch, hydroxypropylcellulose, microcyrstalline cellulose, croscarmellose sodium, talc,

hydrogenated vegetable oil and magnesium stearate, macrogol 6000, polyvinyl alcohol, macrogol 3350, talc, iron oxide yellow (E172) and titanium dioxide (E171). The second mixture contained 20 mg cis (Z) - Flupentixoldecanoate and thin vegetable oil (triglycerides, medium chain).

The synthetic mixture containing 20 mg Citalopram Hydrobromide equivalent to Citalopram, Lactose monohydrate, Maize Starch, Microcrystalline Cellulose, Glycerol, Copovidone, Croscarmellose Sodium, Magnesium Stearate, titanium dioxide E171, Macrogol) was prepared.

15 mg of Buspirone along with various excepients including; colloidal silicon dioxide, lactose, magnesium stearate, microcrystalline cellulose, and sodium starch glycolate were mixed to form a synthetic mixture.

Two synthetic mixtures containing Fluoxetine along with various excepients, were prepared. The first mixture contained 10 mg fluoxetine, pregelatinised starch. gelatin, patent blue V (E131), yellow iron oxide (E172) titanium dioxide (E171), sodium lauryl sulfate, D&C Yellow No. 10, FD&C Blue No. 2, hypromellose acetate, sucrose, sugar spheres, talc, titanium dioxide and triethyl citrate. The second mixture contained 10 mg Fluoxetine, crospovidone, hypromellose, magnesium stearate, maize (corn) starch, microcrystalline cellulose, polyethylene glycol, silica colloidal anhydrous, and titanium dioxide.

Synthetic mixture for each drug dosage forms was extracted with 100 ml Acetonitrile, filtered, and the first 10.0 ml of the filtrate was rejected.Aliquots of the filtrate were diluted with the same solvent to obtain serial dilutions in concentrations ranging from 10 to 50 µg/ml, 10 to 35 µg/ml, 0.5 to 10 ng/ml, 0.1 to 4 µg/ml, 0.3 to 7 µg/ml and 0.05 to 0.55 µg/ml of I, II, III, IV, V and VI respectively.

5. Preparation of pharmaceuticals stock solutions

The mixed contents of 20 tablets were accurately weighed and finely powdered. Apportion of the powder, equivalent to one tablet of Amisulpride, Flupentixol, Citalopram, Buspirone and Fluoxetine were weighed and diluted to a 100 ml with Acetonitrile. Aliquots of the resulting solutions were diluted with the same solvents to obtain (15 to 35 µg/ml), (0.5 to 10 ng/ml), (0.1 to 4 µg/ml), (0.3 to 7 µg/ml)and (0.05 to 0.55 µg/ml)of Amisulpride, Flupentixol, Citalopram, Buspirone and Fluoxetine respectively.

The mixed contents of 20 capsules were accurately weighed. Apportion of the powder, equivalent to one capsule of Ethosuximide or Fluoxetine was weighed and diluted with Acetonitrile to a 100 ml. The resulting solutionswere filtered and aliquots of each filtrate were diluted with the same solvents to obtain (0.05 to 0.55 µg/ml) and (1 to 17 µg/ml) of Ethosuximide and Fluoxetine respectively.

An equivalent measured volume of injection equivalent to (1 mg) of Flupentixol was diluted with Acetonitrile to 100 ml. Aliquot of this solution was diluted with the same solvent to obtain (0.5 to 10 ng/ml) of Flupentixol.

An equivalent measured volume of syrup equivalent to (1 mg) of Ethosuximide and Citalopram were diluted with Acetonitrile to 100 ml. Aliquots of these solutions were diluted with the same solvent to obtain (10 to 50 µg/ml) and (0.05 to 0.55 µg/ml) of Ethosuximide and Citalopram respectively.

6. Procedures

1 ml of DDQ, CL or TCNQ was added to 1 ml of each drug standard solution, assay solution of pharmaceutical preparations and assay solution of spiked human plasma samples and transferred to 10.0 ml screw capped test tube. The mixtures were set aside at room temperature for indicated time and then transferred to 10.0 ml volumetric flask and the resulting solution was adjusted to volume with specified solvent and measured at 540, 840 and 430 nm for DDQ, TCNQ and CL respectively.

RESULTS AND DISCUSSION

1. Optimization of experimental conditions

Optimal concentration of DDQ, TCNQ and CL, reaction time (RT) for charge transfer complexation with Ethosuximide (I), Amisulpride (II), Flupentixol (III), Citalopram (IV), Buspirone (V) and Fluoxetine (VI) were determined as shown in (Figure 2, 3 and 4 &Table 1). The colorimetric measurements were performed against reagent blank experiments.

Table 1: Optimal conditions for the spectrophotometric analysis of Ethosuximide (I), Amisulpride (II), Flupentixol (III), Citalopram (IV), Buspirone (V) and Fluoxetine (VI) using charge transfer complexation

Drug	Concentration	DDQ conc. (µg/ml)	CHA conc. (µg/ml)	TCNQ conc. (µg/ml)	Reaction time with DDQ (min)	Reaction time with CHA (min)	Reaction time with TCNQ (min)
Ethosuximide	25 µg/ml	30	18	NA	20	25	NA
Amisulpride	30 µg/ml	25	24	40	35	30	30
Flupenthixol	10 ng/ml	30	15	NA	25	35	NA
Citalopram	5 µg/ml	15	NA	NA	40	NA	NA
Buspirone	10 µg/ml	30	24	NA	35	30	NA
Fluoxetine	1 µg/ml	NA	NA	24	NA	NA	45

Effect of temperature

The effect of temperature on the formed CT complexes was studied in the range of 10–60◦C. All the formed complexes were stable up to 40◦C; On the contrary, at temperature higher than 40◦C, the relative intensity decreases due to dissociation of the complexes at higher temperatures. Therefore, the determination of studied drugs was carried out at 25±2◦C.

Effect of organic solvent

Both polar and non-polar solvents such as chloroform, acetone, methanol and Acetonitrile were used to select elegant solvent for the analysis of the drugs. Acetonitrile is found to be suitable solvent as it produces maximum

Spectral characteristics of Ethosuximide (I), Amisulpride (II), Flupentixol (III), Citalopram (IV), Buspirone (V) and Fluoxetine (VI) in these different solvents are compared. The Experimental results indicated that, Acetonitrile gave the maximum absorbance intensity and the most stable complex for studied drugs as shown in (Figure 2).

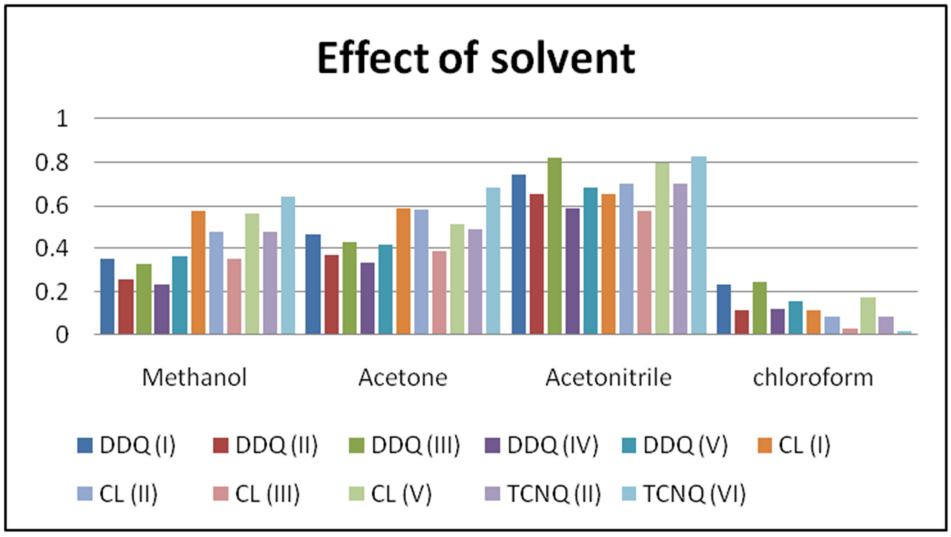

Figure 2: Effect of solvent on the charge transfer spectrophotometric intensity of the reaction of DDQ, CL and TCNQ with Ethosuximide (I), Amisulpride (II), Flupentixol (III), Citalopram (IV), Buspirone (V) and Fluoxetine (VI)

Effect of reagent concentration

The influence of the CT reagents on the relative spectrophotometric intensity of all the formed CT complexes with studied drugs (Ethosuximide (I), Amisulpride (II), Flupentixol (III), Citalopram (IV), Buspirone (V) and Fluoxetine (VI)) was studied at their respective maxima using TCNQ, DD and CL as model electron acceptors. The influence of CT reagent concentration was studied in the range 5 to 35 µg/ml. The relative intensity increased with increasing reagent concentration up to 25µg/ml but leveled off at higher concentrations as shown in (Figure 3).

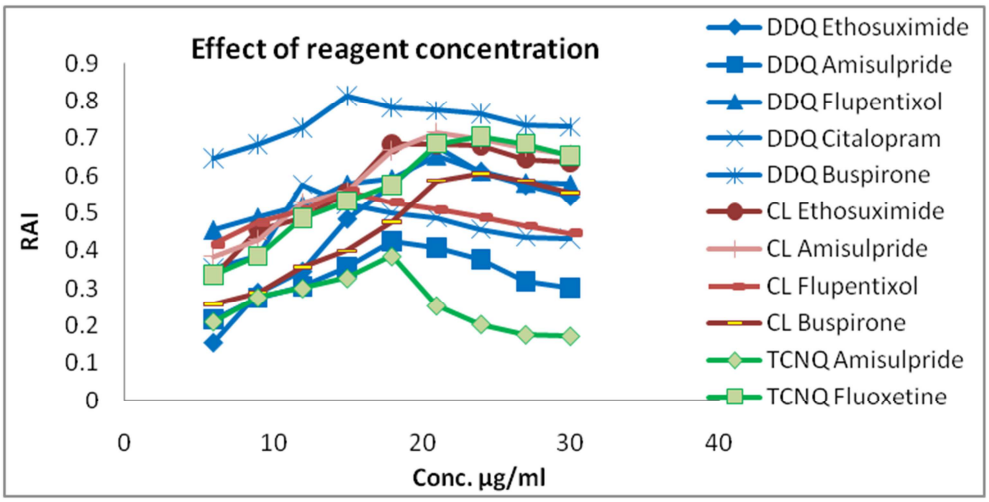

Figure 3: Effect of DDQ, CL and TCNQ concentration (µg/ml) on the charge transfer spectrophotometric intensity of the reaction with Ethosuximide (I), Amisulpride (II), Flupentixol (III), Citalopram (IV), Buspirone (V) and Fluoxetine (VI)

Effect of reaction time

The interaction of DDQ, CL and TCNQ with the investigated drugs resulted in the formation of colored product which stabilized within 20 min. The developed color remained stable at room temperature for about 2 hours. After 1day many solutions turned black and are opaque. All solutions then decolorized hence the measurements were made immediately after mixing the solutions (Figure 4)

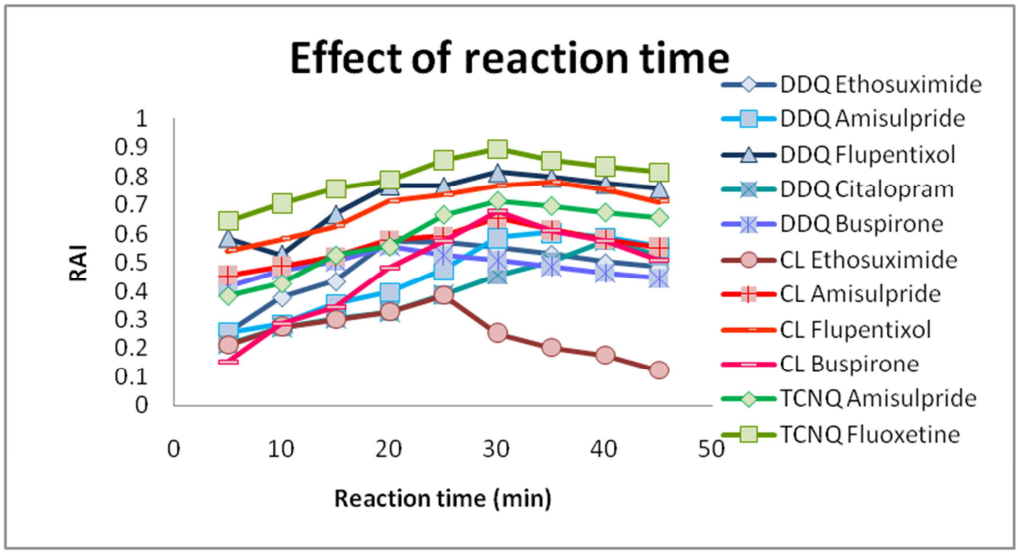

Figure 4: Effect of reaction time on the charge transfer spectrophotometric intensity of the reaction of DDQ, CL and TCNQ with Ethosuximide (I), Amisulpride (II), Flupentixol (III), Citalopram (IV), Buspirone (V) and Fluoxetine (VI)

It can be seen that the solutions of all drugs except Fluoxetine give strong peaks with DDQ at wavelength ranged from (540 to 560 nm). However, in the presence of TCNQ only Amisulpride and Fluoxetine give absorbance peaks which moved to longer wavelengths of 866, 843 nm respectively. The reaction with CL gives dark purple color with highest molar absorptivity (ε) with all studied drugs except Citalopram and Fluoxetine at wavelengths ranged from (430-450 nm).

Having optimized the reaction conditions, the characteristics of the reaction of DDQ, TCNQ and CL with Ethosuximide (I), Amisulpride (II), Flupentixol (III), Citalopram (IV), Buspirone (V) and Fluoxetine (VI) were determined using synchronous wavelength search as shown in (Figure 5, 6 and 7).

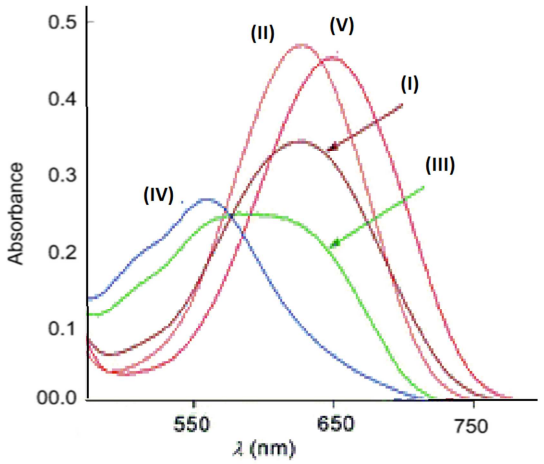

Figure 5: Spectrophotometric spectrum for the reaction of Ethosuximide (I), Amisulpride (II), Flupentixol (III), Citalopram (IV) and Buspirone (V) with DDQ

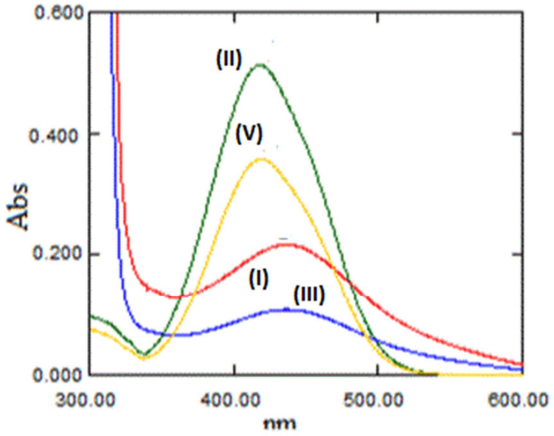

Figure 6: Spectrophotometric spectrum for the reaction of Ethosuximide (I), Amisulpride (II), Flupentixol (III) and Buspirone (V) with CL

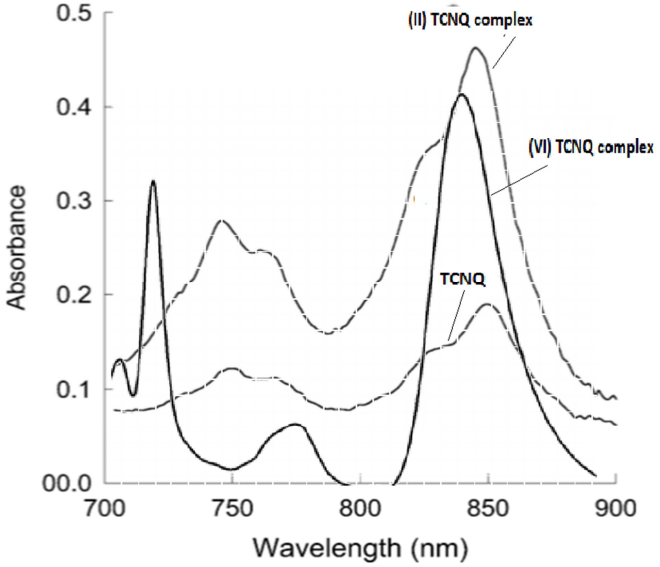

Figure 7: Spectrophotometric spectrum for the reaction of Amisulpride (II), Fluoxetine (VI) with TCNQ

The chromogen formed with DDQ in Acetonitrile is the blood red colored radical anion, which exhibits strong absorption maxima at 490-540 nm (Figure IV). These bands may be attributed to the formation of the radical anion DDQ^{-}, which was probably formed by the dissociation of donor-acceptor (D-A) complex with drugs. The dissociation of the complex was promoted by the high ionizing power of Acetonitrile

Chloranilic acid (p-CL) exists in three ionic forms, the neutral yellow-orange H_2A at very low pH, the dark purple HA^{-} which is stable at pH = 3 and a pale violet A^{2-}, which is stable at high pH; these transformations are illustrated in the following scheme:

$$H_2A \rightleftharpoons H^+ + HA^- \text{ (violet)}$$

$$HA^- \rightleftharpoons H^+ + A^{2-} \text{ (colorless)}$$

Since the interaction of drugs with p-CL in Acetonitrile gave a violet product (Figure 6), it might be concluded that HA^{-} was the form of p-CL involved in the reaction described herein

The predominant chromogen with TCNQ in Acetonitrile is the bluish-green colored radical anion, which exhibits strong absorption maxima at 760-840 nm (Figure 6). These bands may be attributed to the formation of the radical

anion $TCNQ^-$, which was probably formed by the dissociation of an original donor-acceptor (D-A) complex with drugs. The dissociation of the complex was promoted by the high ionizing power of Acetonitrile [38].

The relative sensitivity of the three acceptors employed in the present analytical work may be attributed to their difference in electron affinities, as well as the conditions employed in the reaction (reagent concentration, reaction time, and solvent). DDQ gave relatively weak molar absorptivity values. This may be explained on the basis of insufficient ionization of these relatively weak π-acceptors that possess lower electron affinities than TCNQ and CL.

2. Regression analysis

Under the above optimized conditions, linear relationships between the concentration of Ethosuximide (I), Amisulpride (II), Flupentixol (III), Citalopram (IV), Buspirone (V) and Fluoxetine (VI) and the spectrophotometric intensity were obtained. The linearity of the method was ascertained in standard solutions and in spiked human plasma samples by regression analysis (Table 2).

Table 2: Regression analysis parameters for determination of Ethosuximide (I), Amisulpride (II), Flupentixol (III), Citalopram (IV), Buspirone (V) and Fluoxetine (VI) with DDQ, CL and TCNQ in Acetonitrile standard solutions and in spiked human plasma samples

	Standard solution						Spiked human plasma samples					
Drug	**Linearity range**	**Slope**		**Intercept**		**R^2**	**Linearity range**	**Slope**		**Intercept**		**R^2**
		Mean	**SE**	**Mean**	**SE**			**Mean**	**SE**	**Mean**	**SE**	
DDQ	10-45 µg/ml	0.009	0.62	0.181	0.47	0.998	12-47 µg/ml	0.009	0.41	0.131	0.34	0.995
(I)	y = 0.009x + 0.181						y = 0.009x + 0.131					
(II)	17-35 µg/ml	0.011	0.17	0.121	0.14	0.997	18-30 µg/ml	0.014	0.04	0.019	0.18	0.998
	y = 0.011x + 0.121						y = 0.014x + 0.019					
(III)	0.5-26 ng/ml	0.017	0.25	0.271	0.42	0.996	2-26 ng/ml	0.016	0.51	0.219	0.35	0.995
	y = 0.052x + 0.418						y = 0.053x + 0.292					
(IV)	5-30 µg/ml	0.016	0.32	0.124	0.37	0.998	8-29 µg/ml	0.017	0.34	0.072	0.33	0.995
	y = 0.016x + 0.124						y = 0.017x + 0.072					
(V)	0.5-5.5 µg/ml	0.096	0.54	0.185	0.22	0.995	0.8-5.3 µg/ml	0.107	0.04	0.155	0.21	0.997
	y = 0.096x + 0.185						y = 0.107x + 0.155					
CL	28-49 µg/ml	0.015	0.24	0.001	0.36		32-53 µg/ml	0.014	0.04	0.014	0.14	0.995
(I)	y = 0.015x + 0.001						y = 0.015x + 0.001					
(II)	13-31 µg/ml	0.23	0.08	0.216	0.105	0.996	14-31 µg/ml	0.016	0.01	0.134	0.33	0.995
	y = 0.016x + 0.216						y = 0.016x + 0.134					
(III)	1-9 ng/ml	0.04	0.35	0.014	0.42	0.998	2.5-9.5 ng/ml	0.048	0.25	0.378	0.48	0.999
	y = 0.100x + 0.014						y = 0.048x + 0.378					
(V)	0.2-5.2 µg/ml	0.104	0.02	0.393	0.03	0.996	1-5 µg/ml	0.1	0.08	0.241	0.54	0.998
	y = 0.104x + 0.393						y = 0.1x + 0.241					
TCNQ	10-30 µg/ml	0.028	0.12	0.101	0.04	0.994	12-28 µg/ml	0.025	0.04	0.074	0.05	0.996
(II)	y = 0.028x + 0.101						y = 0.025x + 0.074					
(VI)	12-57 ng/ml	0.01	0.1	0.099	0.07	0.999	15-51 ng/ml	0.014	0.05	0.002	0.17	0.997
	y = 0.010x + 0.099						y = 0.014x + 0.002					

3. Method validation

Various parameters affecting the sensitivity and other validation criteria of the developed method are materialized using the stipulation of ICH Q2B (R1) [39].

3.1. Detection limit (DL) and quantitation limit (QL)

DL and QLare expressed as

$$DL = \frac{3.3\,\sigma}{S} \qquad QL = \frac{10\,\sigma}{S}$$

Where: (σ) is the standard deviation of the response for blank Experiment and S is the slope of the calibration curve.Using the above equations we calculated DL and QL and compared with that experimentally determined ones both in standard solution and spiked human plasma as shown in table 3.

Table 3: Calculated and determined detection limits and quantitation limits of Ethosuximide (I), Amisulpride (II), Flupentixol (III), Citalopram (IV), Buspirone (V) and Fluoxetine (VI) with DDQ, CL and TCNQ in acetonitrile standard solutions and in spiked human plasma samples

Drug		DL						QL					
		(I)	**(II)**	**(III)**	**(IV)**	**(V)**	**(IV)**	**(I)**	**(II)**	**(III)**	**(IV)**	**(V)**	**(IV)**
DDQ (s)	cal.	8.2	15.9	0.38	3.4	0.24	NA	9.5	17.2	0.57	4.8	0.43	NA
	Exper.	8.5	15.4	0.42	3.8	0.2		10	17	0.5	5	0.5	
DDQ p)	cal.	10.4	16.7	0.45	6.5	0.62		11.7	17.8	1.4	8.2	0.74	
	ExP	10	17	1	6.7	0.5		12	18	2	8	0.8	
CHA(s)	cal.	27.5	11.8	0.2	NA	0.08	NA	27.1	13.3	1.4	NA	0.12	NA
	Exp	27	12.1	0.5		0.1		28	13	1		0.2	
CHA (p)	cal.	31	12.9	1.2		0.62		31.6	13.7	2.8		1.3	
	Exp	30.3	13.4	1.6		0.5		32	14	2.5		1	
TCNQ(s)	cal.	NA	9.1	-----	NA	NA	10.8	NA	9.6	NA	NA	NA	11.8
	Exp		9.5				10.5		10				12
TCNQ (p)	cal.		11				14.6		12.2				14.6
	Exp		11.4				14		12				15

The difference between the practically determine DL and QL values and the calculated ones might be ascribed to the limitations built in the particular instrument used. Further, the higher values of detection and quantitation limits in case of spiked human plasma samples might be rationalized on the basis of possible partial binding of the drug to plasma components which makes the bound part unavailable.

Based on the above DL and QL limits and peak plasma concentrations of all available dosage forms the developed method would be suitable for monitoring the blood level of these drugs in patients after administration of a single dose of each dosage form.

3.2. Accuracy

The mean percentage recovery of triplicate determinations of the reaction mixtures of Ethosuximide (I), Amisulpride (II), Flupentixol (III), Citalopram (IV), Buspirone (V) and Fluoxetine (VI) with DDQ, CL and TCNQ in Acetonitrile standard solutions and in spiked human plasma samples using different concentrations lying in the linearity range of each was determined as shown in table 4.

Table 4: Mean values of accuracy parameters of Ethosuximide (I), Amisulpride (II), Flupentixol (III), Citalopram (IV), Buspirone (V) and Fluoxetine (VI) with DDQ, CL and TCNQ in standard solutions and in spiked human plasma samples

D	**Mean**	**DDQ (S)**	**DDQ (P)**	**CL (S)**	**CL (P)**	**TCNQ (S)**	**TCNQ (P)**
I	%R	99.164	99.788	99.662	99.648	NA	NA
	S.D.	0.489	0.470	0.350	0.957		
	C.V.	1.824	1.684	0.890	0.377		
II	%R	100.928	99.680	99.922	101.001	99.587	97.902
	S.D.	0.629	0.349	0.317	0.297	0.345	0.297
	C.V.	2.543	1.489	1.490	1.311	1.960	1.435
III	%R	99.832	100.976	102.558	99.425	NA	NA
	S.D.	0.174	0.188	0.094	0.170		
	C.V.	7.852	3.989	2.494	3.447		
IV	%R	NA	NA	NA	NA	98.236	100.047
	S.D.					0.5139	0.499
	C.V.					3.5998	2.813
V	%R	101.563	101.745	100.156	101.747	NA	NA
	S.D.	0.0691	0.0724	0.075	0.092		
	C.V.	4.431	3.076	5.475	3.357		
VI	%R	NA	NA	NA	NA	100.013	99.832
	S.D.					0.739	0.361
	C.V.					2.729	1.313

The excellent mean %recovery values, close to 100%, and their low standard deviation values represent high accuracy of the analytical methods. The range of mean recoveries was found to be 98.236% (±0.0691) to 101.745% (±0.957) for standard solutions and spiked human plasma samples. These results indicate an agreement between the true values of the prepared concentrations and the values found practically (Table 4).

3.3. Precision

The precision of the method was judged by performing intra-day and inter-day (three days intervals) analyses of different concentrations covering the linearity range in both standard solution and spiked human plasma samples. The results are expressed as **S.D.** and **C.V**. as shown in (Table I& II supplementary information).The range of standard deviation (SD) and coefficients of variation (CV %) was found to be from 0.037 to 7.259 and from 0.259 to 15.301% for SD and CV respectively in both standard solution and spiked human plasma samples T**he small CV% and SD indicate high precision of our method.**

3.4. Interferences

Interferences are compounds that have the ability to form CT complexes. Thus as far as drugs are concerned, other antipsychoticsgiving positive reaction due to the formation of such complexes. However, such compounds are not usually present with examined drugs, and hence are not likely to cause analytical problems. On the other hand, tablet excipients represent a potential source of interference. Therefore synthetic mixtures containing drugs along with the excipients used during pharmaceutical formulations were prepared. These mixtures were analyzed using the proposed method and the results, were expressed as % recovery ± S.D. (Table 5).

Table 5: Mean specificity parameters of Ethosuximide (I), Amisulpride (II), Flupentixol (III), Citalopram (IV), Buspirone (V) and Fluoxetine (VI) with DDQ, CL and TCNQ in synthetic mixtures

First mixture						
Drug	I	II	III	IV	V	VI
DDQ						
Mean %R	99.832	99.500	99.489	98.684	99.839	
S.D. ±	0.361	0.286	0.058	0.307	0.224	NA
C.V. %	1.313	3.772	2.885	0.311	0.224	
CHA						
Mean %R	100.024	99.568	99.948		99.704	
S.D. ±	0.421	0.241	0.075	NA	0.230	NA
C.V. %	1.143	2.263	3.104		0.231	
TCNQ						
Mean %R		99.720				99.823
S.D. ±	NA	0.253	NA	NA	NA	0.368
C.V. %		2.190				0.369

Second mixture			
Drug	I	III	VI
DDQ			
Mean %R	99.977	99.379	
S.D. ±	0.352	0.086	NA
C.V. %	0.896	3.152	
CL			
Mean %R	99.129	99.859	
S.D. ±	0.297	0.072	NA
C.V. %	3.3059	2.755	
TCNQ			
Mean %R			100.334
S.D. ±	NA	NA	0.332
C.V. %			0.331

The above results indicate good selectivity of the method to determine the studied drugs both in raw material and in their dosage forms.

4. Analysis of commercial formulations

The proposed methods have been applied for the analysis of drugs commercial tablets, capsules, injections and drops according to the official USP 24 and BP. The results were expressed as % recoveries ± S.D (Table 6). The experimental values did not excess the theoretical values and all the % recoveries meet the pharmacopeial limits, which indicate that the method is highly specific and applicable for drugs dosage forms.

5. Stoichiometry

The Job's method of continuous variation [34] was employed. Master equimolar solutions of each drug and reagents were prepared. The concentrations of these solutions were 4.9×10^{-1} M for TCNQ, 38.8×10^{-1} M for DDQ and 39×10^{-1} M for p-CL in Acetonitrile. Series of 10-ml portions of the master solutions of each drug with the respective reagent were made up comprising different complementary proportions (0:10, 1:9, ……, 9:1, 10:0, inclusive) in 10-mL calibrated flasks. The reactions were allowed to proceed for the optimum reaction time (Table I) and then the absorbance of the resulting solutions was measured at the corresponding wavelengths of maximum absorbance (λ_{max}).

Table 6: Recovery data of Ethosuximide (I), Amisulpride (II), Flupentixol (III), Citalopram (IV), Buspirone (V) and Fluoxetine (VI) with DDQ, CL and TCNQ in their pharmaceutical preparation

Drug	Pharmaceutical preparation	%Recovery ±SD
Ethosuximide	1. Zarontin 250 mg caps 2. Zarontin 250 mg /5 ml syrup	DDQ
		100.57% ± 1.88 101.78% ± 1.346
		CHA
		99.15% ± 0.566 101% ± 0.26
Amisulpride	1. AMIPRIDE 50mg tablet	DDQ
		100.85% ± 0.66
		CHA
		100.72% ± 0.09
		TCNQ
		99.85%± 0.39
Flupenthixol	1. Fluanxol 3 mg tab 2. Fluanxol depot 40 mg /2 ml ampoule	DDQ
		100.31± 0.54 101.38% ±1.86
		CHA
		101% ± 0.76 101.24%±1.82
Citalopram	1. Citalo 20 mg f.c.tablet 2. Citalo 2mg /ml syrup	DDQ
		99.57%±0.058 102.53%±0.17
Buspirone	Exupar 15 mg tablet	DDQ
		99.78%±0.38
		CHA
		99.86%±0.41
Fluoxetine	1. Fluozac 40 mg capsule 2. Fluoxetine 20 mg capsule 3. Durazac 90 mg delayed release cap. 4.Prozac 20 mg tablet	TCNQ
		101.36%±0.37 102.28%±0.028 98.91%±0.037 102.21%±0.056

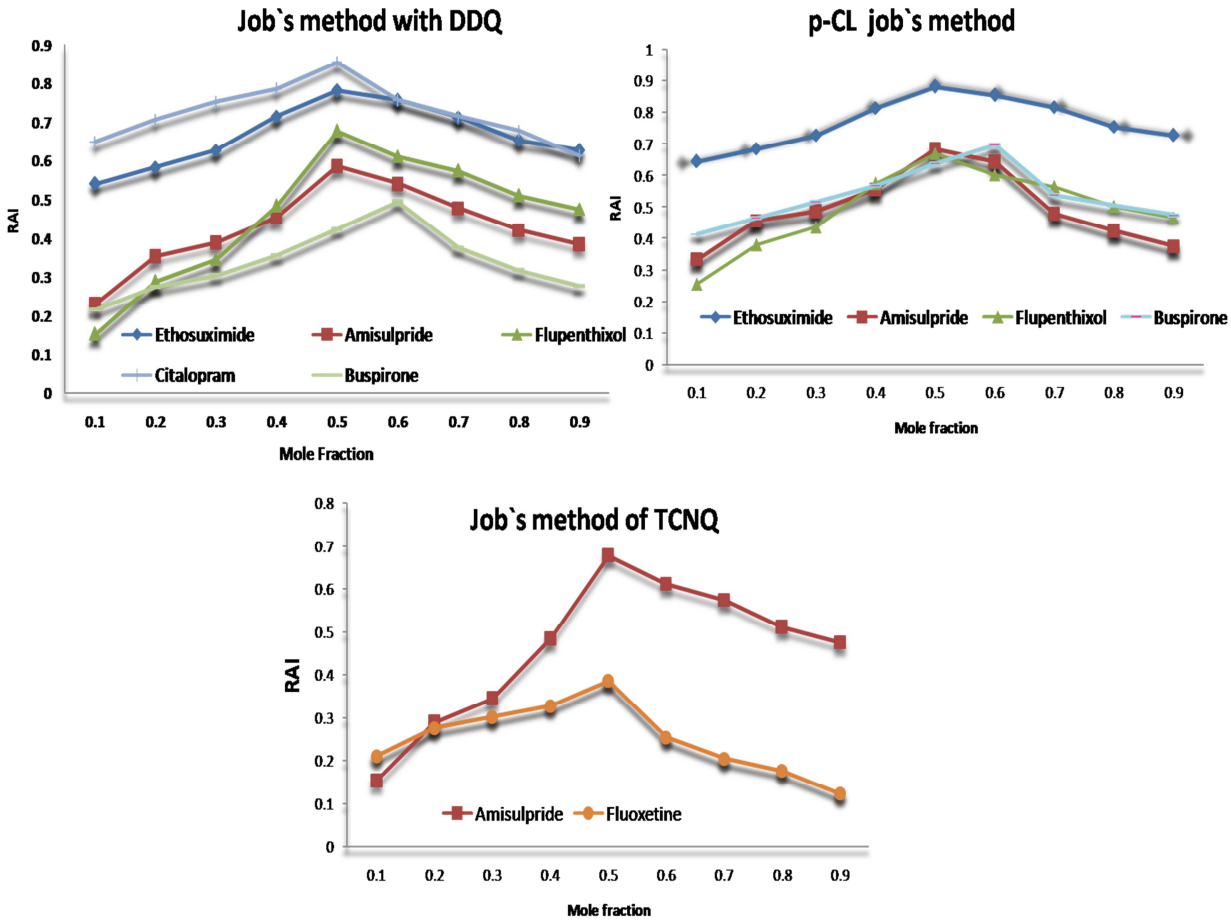

Figure 8: The Stoichiometry of the complexes of DDQ, CL and TCNQ with (I), Amisulpride (II), Flupentixol (III), Citalopram (IV), Buspirone (V) and Fluoxetine (VI) determined by Job's method

The results indicated that interaction of all the drugs with DDQ, CL and TCNQ occurs on equimolar basis. The reaction was postulated to proceed as 1:1 ratio for DDQ, CL and TCNQ with all drugs except Buspirone via only one site of interaction in spite of the presence of more than one possible electron-donating site. For Buspirone the ratio was 1:2 this indicated that two moles of DDQ, CL and TCNQ interacted with one mole of Buspirone as shown in Figure 8.

The stability constants of the formed charge transfer complexes of DDQ, CL and TCNQ with (I), Amisulpride (II), Flupentixol (III), Citalopram (IV), Buspirone (V) and Fluoxetine (VI) were calculated according to the Benesi–Hildebrand equation [40].

$$\frac{[A]}{A_{CT}} = \frac{1}{K_{CT}\varepsilon_{CT}[D]} + \frac{1}{\varepsilon_{CT}}$$

Where[D] is the molar concentration of the donor, [A] is the sum of the reagent concentration in the complex and in the free State, A_{CT}, absorbance of the formed complex, K_{CT} association constant and ε_{CT} are the molar absorptivity of the formed complexas shown in Table 7.

Table 7: Stability constant (Kct) values of the formed complexes after reaction of Ethosuximide (I), Amisulpride (II), Flupentixol (III), Citalopram (IV), Buspirone (V) and Fluoxetine with DDQ, CL and TCNQ which are determined by Benesi–Hildebrand method.

Drug	DDQ		CHA		TCNQ	
	ε CT *10^3	kct *10^3	ε CT *10^3	kct *10^3	ε CT *10^3	kct *10^3
(I)	2.86	1.07	2.83	1.549	NA	
(II)	2.74	2.26	2.3	1.739	5.4	0.500
(III)	4.61	2.085	5.2	0.769	NA	
(IV)	11.49	0.512	NA			
(V)	3.14	4.19	2.5	2.222	NA	
(VI)	NA				2.8	1.984

6. Investigations on the structure of the CT complexes

DDQ, CL and TCNQ are π-acceptors; Ethosuximide (I), Amisulpride (II), Flupentixol (III), Citalopram (IV), Buspirone (V) and Fluoxetine (VI) are nitrogenous compounds. These drugs were probably through the lone pair of electron donated by the N atom [in pyrrolidine ring of I and II, pyrimidine ring of V, piperazinyl ring of III and V and peripheral tertiary or secondary nitrogen atom of IV and VI respectively (n-electron donors)] to DDQ, CL and TCNQ (π-electron acceptor). So, CT complexes can be formed with these drugs. The distinct appearance of colorimetric peak with high intensity indicated the possible CT complexes formation of the type n–π complexes. The formation of such complexes was also confirmed by both IR and UV measurements. The majority of infrared measurements on such CT complexes have been concerned with the shifts in the vibrational frequencies of donors or acceptors. Decreases in the vibration frequency of a particular band have been used as an evidence for a particular site of a CT interaction [41]. The infrared spectra of the complexes shows some differences compared with the sum of the spectra of the two components.

6.1. Preparation of the complexes for infrared

To 5ml of 0.1mol l^{-1} of CL in methanol and 5ml of 0.1mol l^{-1} of each investigated drug in methanol was added in around bottom flask containing 50ml of methanol and stirred for 30min for 8 hours. The solvent was evaporated under reduced pressure and the resulting residues were dried over calcium chloride.

6.2. Physical measurements of the separated complexes

A- Melting point

Melting point analysis was useful for differentiating the chemically synthesized CL-Drug products from the reactants; though melting points of the separated CT complexes of the studied drugs (I, II, III and V) with chloranilic acid were determined on Griffin melting points apparatus and compared with those of free drugs. The results are presented in Table 8.

Table 8: The melting points for the investigated CT complexes of Ethosuximide (I), Amisulpride (II), Flupentixol (III) and Buspirone (V) with CL

Drug	Molecular formula	Melting point	
	CT complex with CL	Reported drug m.p	CT complex with CLm.p
(I)	$C_{13}H_{13}NCl_2O_6$	64.5- 65.5 °C	182-183°C
(II)	$C_{23}H_{29}N_3Cl_2O_8S$	126 - 127°C	212-213°C
(III)	$C_{29}H_{27}F_3N_2Cl_2O_5S$	233-234 °C	271-272°C
(V)	$C_{27}H_{33}N_5Cl_2O_6$	201.5-202.5 °C	254-255°C

The distinct change of the melting points of the studied drugs with CL indicates the possible CT complexes formation.

B- Microanalytical study

Microanalysis was carried out with Perkin Elmer model 2400 series II CHNS/O elemental analyzer in the Department of Chemistry, Cairo University, Egypt.

Elemental analysis is besides the established methods of structure elucidation (MS, NMR, IR and other spectral methods) very important analytical methodology for correct characterization of prepared substance.

The purity and contribution of elements (CHN) of the synthesized complexes of the studied drugs (I, II, III and V) with CL were checked by the elemental analysis and the results are tabulated in table 9.

The data analyzed indicate that the experimentally obtained values (within the bracket) were in good agreement with theoretical values. The result confirms the formation of the compound in Stoichiometric proportion and the compound is free of impurities.

Table 9: Microanalysis of CT complexes of CT complexes of Ethosuximide (I), Amisulpride (II), Flupentixol (III) and Buspirone (V) with CL

Drug	Molecular weight	Microanalysis calculated (found) CL complex		
		C	H	N
(I)	350.15	44.59 (44.88)%	3.74 (3.22)%	4.00 (4.35)%
(II)	578.46	47.76 (47.45)%	5.05 (5.13)%	7.26(7.54)%
(III)	643.50	54.13 (53.83)%	4.23 (4.43)%	4.35 (4.81)%
(V)	594.49	54.55 (54.47)%	5.59 (5.87)%	11.78 (11.44)%

C- IR Spectra

The IR spectrum of chloranilic acid exhibits two asymmetric and symmetric stretching frequencies at 1670 and 1630 due to the two CO groups and shows strong bans at 1540, and 860cm^{-1} corresponding to aromatic C=C and 1,4-disubstituted benzene stretching, respectively.

The IR spectrum of those complexes shows two CO stretching bands at 1638 and 1546 cm^{-1}. These carbonyl band shifts suggested the formation of hydrogen bond with the investigated drugs I, II, III and V by only one CO.The IR spectra of the complexes also are characterized by a broad medium band that appears between 2400-2800 cm^{-1}, which does not appear in the spectra of the free donors or those of the CL acceptor. These broadened peaks can be attributed to the stretching vibration of the intermolecular hydrogen bond in the complex formed through the transfer of a proton from the acidic center of CL acceptor to the donors.

The peaks due to C=O stretching of (I, II &V) and OH stretching of (II) are now shifted to lower cm^{-1}, which implies that these groups are participating in a strong hydrogen bond. The peaks of secondary and tertiary nitrogen atoms cyclic or openchain are also shifted to a lower wave number due to CT complexes of all drugs as shown in table (Table 10).

Table 10: Comparative FT-IR data of free investigated drugs (I, II, III and V), free CL and CT complexes [42]

Name of the compound	Characteristic peaks cm^{-1}	Shifted groups at complexes	Corresponding functional group
Ethosuximide	3642.35 1700 -1777 1303 Absent	3568.84 1658 2400-2800 cm^{-1}	N-H (CO) stretching Symmetric C=O stretching CH_2 bend intermolecular hydrogen bond stretching
Amisulpride	3412.52 1690 1356 1457.0, 1486.96 Absent	 1573.21 1378.54-1411.56 2400-2800 cm^{-1}	Amine stretching C=O amide stretching Sulfone group stretching Pyrrole ring intermolecular hydrogen bond stretching
Flupentixol	3000-3100 and 1400-1600 3200-3650 1000-1400 2850-3000 1200-1350 Absent	 2985-3000 980-1100 2400-2800 cm^{-1}	Aromatic ring skeleton vibration. –OH group. indicates C-F present C-H bond stretching. Tertiary amine group Intermolecular hydrogen bond stretching
Buspirone	1650–1700 1500–1600 3000–3100 1875-1780 1200-1300 Absent	1574-1600 1798-1820 1000-1050 2400-2800 cm^{-1}	C=O stretching C=C stretching C-H in aromatic ring C=N stretching Tertiary amine group intermolecular hydrogen bond stretching
Chloranilic acid	1670 and 1630 1368.86 689.13, 751.61 1540 860 Absent	1540 and 860 1254.32 2400-2800 cm^{-1}	1.4 quinone Tertiary alcohol –C–Cl bond Aromatic C=C 1,4-disubstituted benzene stretching intermolecular hydrogen bond stretching

From data obtained from job`s mole fraction method, melting points, elemental analysis and IR spectra interpretation we have suggested the mechanism of reaction of CL with Ethosuximide (I), Amisulpride (II), Flupentixol (III) and Buspirone (V) as shown in figure (9)

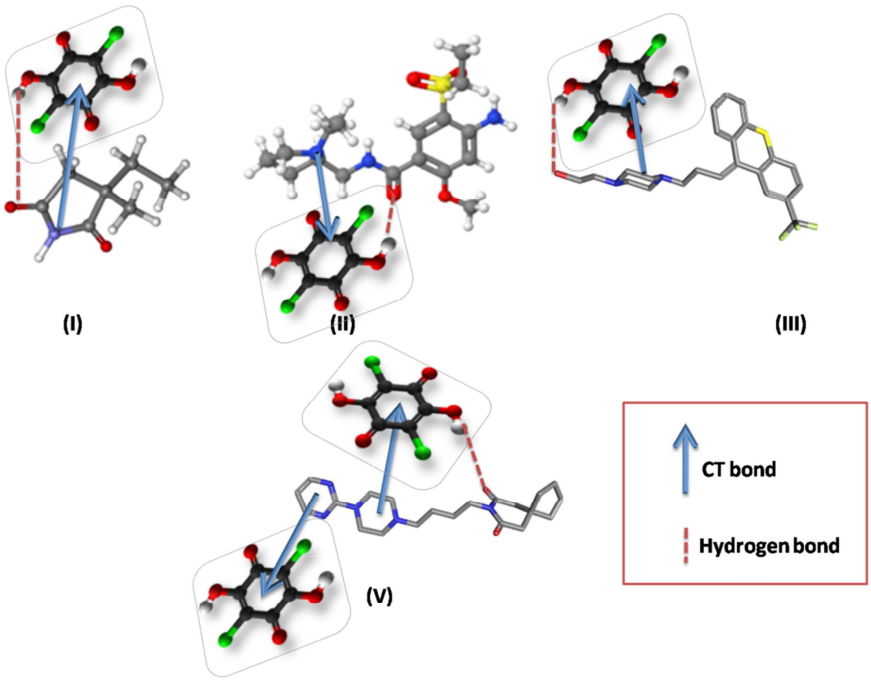

Figure 9: The structure of chloranilic acid complexes withEthosuximide (I), Amisulpride (II), Flupentixol (III) and Buspirone (V)

CONCLUSION

The suggested method has the advantage of being simple, accurate, sensitive and suitable for routine analysis in control laboratories. The P-CL method was more sensitive than the other methods due to the higher molar

absorptivity. These methods can be used as general methods for the spectrophotometric determination of Ethosuximide (I), Amisulpride (II), Flupentixol (III), Citalopram (IV), Buspirone (V) and Fluoxetine (VI). The proposed methods are suitable for the routine quality control of the drugs alone and in tablets, oral drops or injection without fear of interference caused by the excipients expected to be present in formulations. The high sensitivity of these methods also permits the determination of the studied drugs in biologic fluids.

The charge-transfer complexes with CL were isolated and characterized using microanalyses and FT-IR. The Stoichiometry of the products was found to be 1:1except for Buspirone it was 1:2. Accordingly, the formed CTcomplexes have the formulas [(I, II or III)(P-CL)] and [(V)(P-CL)$_2$].

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