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Research Article

# SPECTOPHOTOMETRIC DETERMINATION OF DISTIGMINE BROMIDE, CYCLOPENTOLATE HCL, DIAVERIDINE HCL AND TETRAHYDROZOLINE HCL VIA CHARGE TRANSFER COMPLEX FORMATION WITH DDQ REAGENT

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**ABSTRACT**

In this work, distigmine bromide, cyclopentolate hydrochloride, diaveridine hydrochloride and tetrahydrozoline hydrochloride were chosen to study their properties from the analytical point of view. The purpose of this investigation was directed to propose sensitive, accurate and reproducible methods of analysis that can be applied to determine these drugs in pure form and pharmaceutical preparations. The studied work with the spectrophotometric determination of distigmine bromide, cyclopentolate hydrochloride, diaveridine hydrochloride and tetrahydrozoline hydrochloride via charge-transfer complex formation. This includes the utility of π-acceptor like 2,3-dichloro-5,6-dicyanobenzoquinon (DDQ) for estimation of distigmine bromide, cyclopentolate hydrochloride, diaveridine hydrochloride and tetrahydrozoline hydrochloride in their pure form and in pharmaceutical preparations.

**Keywords:** distigmine bromide, cyclopentolate hydrochloride, diaveridine hydrochloride**.**

## INTRODUCION

Analytical methods that were used for the quantitative determination of drugs played a significant role in the evaluation and interpretation of bioavailability, bioequivalence, pharmacokinetic and fully validated analytical methods to yield reliable results that could be satisfactory interpreted. Analytical methods and techniques were constantly being changed and improved, in many instances, these methods were at cutting edge of the technology. Also, it was important to emphasize that each analytical technique had its own characteristics, which will vary from drug to drug.

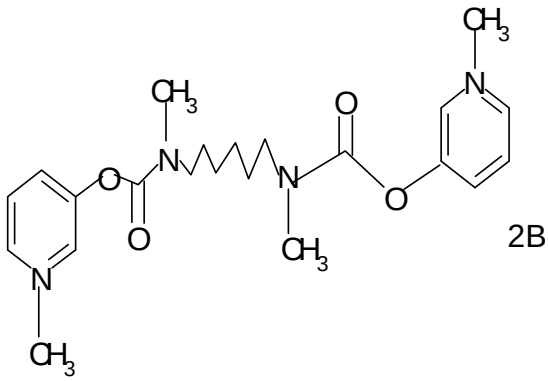
### Literature survey on distigmine bromide

Distigmine bromide was designated by chemical abstracts as:

3,3-[hexamethylene bis(methylimino-carbonyloxy)]bis(1-methylpyridinium) dibromide.

It has the molecular formula $C_{22}H_{32}Br_2N_4O_4$ (F.W 576.33 g/mole).

It has the following structure.



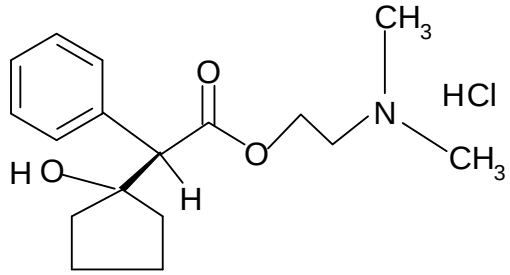

**Structure of distigmine bromide**

Distigmine bromide, calculated on the anhydrous state, contains not less than 98.5% of distigmine bromide ($C_{22}H_{32}Br_2N_4O_4$).
It occurs as a white crystalline powder, slightly hygroscopic and soluble in water, freely soluble in methanol, ethanol and chloroform, and practically insoluble in acetone and ether. It becomes odourless, and gradually coloured on exposure to light. The pH of a solution of distigmine bromide (1:100) is between 5.0 and 5.5.
It was identified according to the previously reported method[1].

**Colourimetric method**
Demecarium bromide was quantitatively determined by colourimetric method based on the reaction with potassium cobaltothiocyanate. The blue colour was extracted with 1,2-dichloroethane and the absorbance was measured at 320 and 625 nm. The method was suitable for use in the presence of possible hydrolytic products of demecarium bromide; a brief stability study of the drug in aqueous solutions is reported. The method was also applied to other structurally related cholinesterase inhibitors such as pyridostigmine bromide and distigmine bromide[2].

**Chromatographic method**
A screening procedure was developed for the identification and quantification of distigmine bromide in serum samples by using liquid chromatography (LC)–electro spray ionization (ESI)-mass spectrometry (MS). In this method, distigmine bromide was analyzed in 0.5 mL serum by using pancuronium bromide as the internal standard, and gradient elution was performed using a reversed-phase column and a mixture of 10 mM-ammonium formate and methanol as the mobile phase. A highly sensitive assay could be performed with simple solid phase extraction using a cation exchange cartridge column by carrying out selected ion monitoring analysis in the positive ion detection mode. The procedure was validated in terms of linearity ($0.997 < r^2 < 0.999$ for concentrations ranging from 5 to 250 µg $mL^{-1}$), extraction recovery (83.0 to 89.3%, n 5), and detection limit (S/N ratio: >3 at 2.5 µg $mL^{-1}$). The inter- and intra-day precisions (coefficient of variation CV%) were <8.5 and <9.7%, respectively. The analytes were evaluated for stability and were found to be stable in serum for 1 week and 4 weeks at 4°C and -30°C respectively, and successfully applied to in the analysis of two overdose cases. This method was sensitive and useful for the detection, quantification, and confirmation of distigmine bromide in serum[3].

**Literature survey on cyclopentolate hydrochloride**
Cyclopentolate hydrochloride was nomenclated according to the IUPAC system as: benzene acetic acid, α-(1-hydroxycyclopentyl)-2-(dimethylamino) ethyl ester, hydrochloride, (±)-. or 2-(dimethylamino) ethyl (±)-1-hydroxy-α-phenylcyclopentaneacetate hydrochloride. It has the molecular formula $C_{17}H_{25}NO_3$.HCl (F.W 327.85 g/mole). the structure was given below.




**Structure of cyclopentolate HCl**

The colour was white or almost white, crystalline powder, hygroscopic, very soluble in water and freely soluble in alcohol. Cyclopentolate hydrochloride contains not less than 98 and not more than 102% of $C_{17}H_{25}NO_3$.HCl, calculated on the dried basis[4]. Cyclopentolate hydrochloride is an anticholinergic drug that blocks the response of the sphincter muscle of the iris and the accommodative muscle of the ciliary body to stimulation by acetylcholine.

**Chromatographic methods**
According to chromatographic technology application note, cyclopentolate enantiomers were separated by HPLC using a CHIRAL-AGP Column (10 cm × 4 mm i.d.) and CHIRAL-AGP guard column (1 cm × 3 mm i.d.), a mobile phase of 4% propan-1-ol in 10 mM – sodium phosphate buffer of pH 7 and detection at 225 nm. The mobile phase flow rate was not specified. A chromatogram is presented[5].
Analysis is performed on a deactivated glass – capillary column (25cm × 0.3mm) coated with SE-30 and operated at 180 degree with splitless injection, N as carrier gas (2 mL $min^{-1}$) and f.i.d. hexadecanol is used as internal standard, and the compounds were silyated with trifluorobis(trimethylsilyl)acetamide–chlorotrimethylsilane (99:1) before injection[6].

**Other methods**

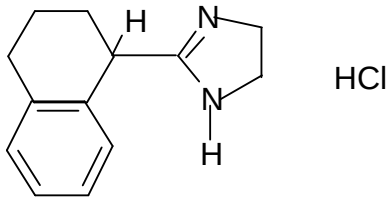
One visible spectrophotometric and another fluorimetric method have been developed for the determination of cyclopentolate hydrochloride from bulk and ophthalmic solutions. Spectrophotometric method was based on the formation of greenish blue coloured species on treatment with Folin-Ciocalteu (FC) reagent in alkaline medium, showing maximum absorbance at 733 nm that obeyed Beer's law in the concentration range 20–240 µg $mL^{-1}$. Fluorimetric method was based on the hydrolysate solution of cyclopentolate hydrochloride in 0.5 sodium hydroxide on excitation at 366 nm emits yellowish blue fluorescence measured at 475 nm, which showed linearity in the concentration range of 20 – 600 µg $mL^{-1}$ [7].

Another method has been developed for the determination of cyclopentolate hydrochloride in pure form and in its pharmaceutical preparations. Aliquots (1-5 mg) of the samples are allowed to react with 0.3 g equivalent / L ammonium metavanadate reagent in acidic medium for the required reaction time at boiling water bath. After the reaction was completed, the unconsumed reagent was back determined by titration against ferrous ammonium sulphate using N phenylanthranilic acid indicator. The value of % error, SD and CV reflect the precision and reproducibility of this method[8].

Four novel ion - exchangers (Fx-Rt (I), Fx-TPB (II), Cp3-PMA (III) and Cp3-PTA (IV)) of antispasmodic and anticholinergic drugs, flavoxate hydrochloride (FxCl), 2-piperidinoethyl-3-methyl-4-oxo-2-phenyl-4h-1-benzopyran-8-carboxylate hydrochloride, cyclopentolate hydrochloride (CpCl) and (2-(dimethylamino)ethyl(RS)–(1-hydroxycyclopentyl)phenylacetate) hydrochloride were synthesized and incorporated into poly(vinyl chloride)-based membrane electrodes for the quantification of FxCl and CpCl in different pharmaceutical preparations. The influence of membrane composition on the potentiometric response of the membrane electrodes was found to substantially improve the performance characteristics. The best performance was reported with membranes having compositions (w/w) of Fx-Rt (2%): PVC (49%): DOP (49%), Fx-TPB (7%): PVC (46.5%): DOP (46.5%), Cp3-PMA (8%): PVC (46%): DOP (46%) and Cp3-PTA (9%): PVC (45.5%): DOP (45.5%). The proposed sensors exhibited Nernstian responses in the concentration ranges of $1.39 \times 10^{-6}$–$5.00 \times 10^{-4}$, $9.90 \times 10^{-7}$–$3.75 \times 10^{-5}$, $1.39 \times 10^{-5}$–$2.53 \times 10^{-3}$ and $3.21 \times 10^{-6}$–$8.62 \times 10^{-4}$ M, with detection limits of $5.50 \times 10^{-7}$, $9.8 \times 10^{-7}$, $9.8 \times 10^{-6}$ and $2.95 \times 10^{-6}$ M for the (I), (II), (III) and (IV) electrodes, respectively. The membrane electrodes performed satisfactorily over pH ranges of 2.0–5.5, 2.0–5.5, 2.0–5.0 and 2.0–7.5, with fast response times of 20, 30, 15 and 20 s for the (I), (II), (III) and (IV) electrodes, respectively. The practical utility of the sensors was demonstrated by the determination of FxCl and CpCl in pure solutions and pharmaceutical preparations using standard additions and potentiometric titration[9].

**Literature survey on tetrahydrozoline hydrochloride**

Tetrahydrozoline hydrochloride has a molecular formula $C_{13}H_{17}CLN_2$ (F.W. 236.7). Its structure was given below.




**Structure of tetrahydrozoline HCl**

It has the IUPAC name 2-[(1RS)-1, 2, 3, 4-tetrahydronaphthalen-1-yl]-4,5-dihydro-1H-imidazole hydrochloride. It contains 98.0 per cent to 101.0 percent dried substance. It is White or almost white, crystalline powder. It is freely soluble in water, anhydrous ethanol and ethanol (96%) and practically insoluble in acetone[10].

**Chromatographic methods**

HPLC method has been developed for the simultaneous determination of ofloxacin (OFX), tetrahydrozoline hydrochloride (THC), and prednisolone acetate (PAC) in ophthalmic suspension using propylparaben (POP) as the internal standard. The mobile phase consists of 0.5 M phosphate buffer/acetonitrile (65:35, v/v), and pH 2.7.

A column containing octadecyl silane chemically bonded to porous silica particales (waters spherisorb, 5 microm ODS 1, 4.6×150 mm) is used as the stationary phase. The detection is carried out using UV – visible detector at 210 nm for OFX and THC and 254 nm for POP (internal standard) and PAC. The solutions are chromatographed at a constant flow rate of 1.2 mL $min^{-1}$. Retention times for OFX, THC, POP, and PAC are approximately 2.5, 4.5, 7.8 and 9.5 min, respectively. The relative retention times are approximately 0.14 min for OFX, 0.35 min for THC, 1.00 min for POP, and 1.22 min for PAC. The linearity

range and percent recoveries for OFX, THC, and PAC are 24-120, 4-16 and 16-80 µg $mL^{-1}$ and 100.48%, 100.34% and 100.21%, respectively [11].

Samples, containing tetrahydrozoline hydrochloride (I), were diluted with mobile phase if required and portions were analysed on a 5 microm LiChrospher Si 60 column with aqueous 70% methanol, containing 0.03% triethylamine and 0.02% acetic acid as mobile phase (1mL $min^{-1}$) and detection at 254 nm. Calibration graphs were linear from 1-20 and 12.5-500 µg $mL^{-1}$ of I, with intra – and inter – day RSD on samples of 1.3 – 11.8%. Average recoveries of 20-50 µg $mL^{-1}$ of I was added to ophthalmic solutions were 98.9-99.9%.The method was not affected by the presence of relatively high concentrations of sulfamethoxazole sodium or methyl paraben [12].

For UV spectrophotometric analysis, eye drop formulations were diluted with mobile phase to bring the concentrations of the two ingredients within the calibration ranges. Second – order derivative spectra were recorded from 210 to 300nm in 1cm cells. the tetrahydrozoline hydrochloride (I) and the fluorometholone (II) were measured at 226 and 282 nm, respectively. Calibration graphs were linear from 5-20 and 20-60 µg $mL^{-1}$, respectively, for I and II. For HPLC analysis, 1 mL of eye drop formulation was treated with lidocaine (internal standard, final concentration 8 µg $mL^{-1}$) and diluted to 25 mL with mobile phase. Portions (20 µL) were analyzed on a 3 µm Partisil 5 ODS column (25 cm×4.6 mm i.d.), with $H_2O$/acetonitrile/methanol (1:5:5) as mobile phase (1.5 mL $min^{-1}$) and detection at 220 nm. Calibration graphs were linear from 3-20 and 10-60 µg $mL^{-1}$, respectively, for I and II. Recoveries of 10-18 µg $mL^{-1}$ of I added to 20 µg $mL^{-1}$ of II and of 40-60 µg $mL^{-1}$ of II added to 10 µg $mL^{-1}$ of I by both methods were in the range 99.1 – 102.8%, with RSD of 0.5 – 1.06%. The results on two formulations by both methods were 99.2-101.6% of the labelled values. Both methods were satisfactory, but the HPLC method gave lower RSD [13].

The nasal preparation containing tetrahydrozoline and added tolazoline hydrochloride(as internal standard) was diluted with aqueous 40% methanol. An aliquot(10 µL) was analysed by HPLC on an RSIL C18 column(15 cm×4.1 mm) operated at 25 degree with 220 nm detection; the mobile phase (1 mL $min^{-1}$) consisted of aq.40 % methanol containing 20 mM-Na octane-1-sulphonate and 10 mM-NN-dimethyloctylamine. The pH of the mobile phase was adjusted to 3.0 by adding $H_3PO_4$. Calibration graphs were rectilinear and the coefficient of variation (n 7) and the average recovery were 1.64 and 101.1%, respectively[14].

The sample was diluted with the mobile phase for analysis on a column (15 cm×4.5 mm) of Hypersil C8. The mobile phase (1.5 mL $min^{-1}$) comprises 10% of 5 mM Na-octanesulphonate, 5mM-$Na_2HPO_4$ (PH 7) in acetonitrile-methanol (1:1). Detection is at 222 nm. 4-dimethylaminobenzaldehdye was used as the internal standard. the coefficient of variation for the determination of 1.03 mg $mL^{-1}$ of antazoline phosphate and 0.5 mg $mL^{-1}$ of tetrahydrozoline hydrochloride were 0.36 and 0.42%, respectively(n=6). Excipients and potential degradation products do not interfere [15].

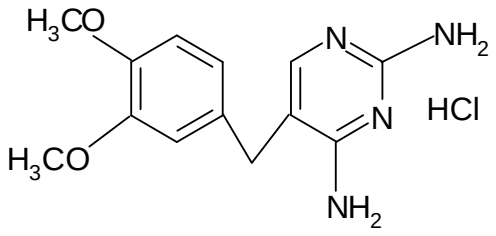
Tetrahydrozoline (I) and its possible decomposition product, N-ethylamino-1, 2, 3, 4-tetrahydro-1-naphthamide (II), were seperated by HPLC on a column (30 cm×3.9 mm) of micro bondapak C18 (10 µm), with $Na_2B_4O_7$-$KH_2PO_4$ buffer solution of pH 7.0 – acetonitrile (3:2) as mobile phase and detection at 254 nm. I and II were found to have a retention times of 5.14 and 3.13 min, respectively. The detection limit was 300 µg of I injected, with rectilinear response in the range from 0.5 to 10 µg. Reproducibility is 1.5%[16].

**Literature survey on diaveridine hydrochloride**

Diaveridine hydrochloride (DVH) was designated by chemical abstracts as: 5-[(3,4-dimethoxyphenyl)-methyl]-2,4-pyrimidinediamine hydrochloride; 2,4-diamino-5- veratrylpyrimidine hydrochloride; 2,4-diamino-5-(3′,4′-dimethoxybenzyl)pyrimidine hydrochloride.

DVH has a molecular weight 296.79 g ⁄ mol and molecular formula $C_{13}H_{17}N_4O_2Cl$ [17].

Its structure was given below.




**Structure of diaveridine HCl**

**Experimental**

**Materials**

All chemicals and reagents were of analytical reagent grade and some of them were used as such without any further purification. These

included distigmine bromide that provided by Arab Drug Company, Reagent used included 2, 3-dichloro-5,6-dicyano-p-benzoquinone (DDQ) supplied from Arcos-USA.
Glacial acetic acid was supplied from Merck. Absolute ethanol was supplied from Adwic, while n-propanol and acetonitrile (AR) were supplied from Aldrich. Chloroform, methanol, acetone, tetrahydrofuran, 1,4–dioxan, n-butanol, methylene chloride and dimethyl formamide were supplied from El-Nasr Company.
The distigmine bromide pharmaceutical preparations were bought from Ubreted tablets, 5 mg / tablet, (Arab Drug Company, Egypt)
The cyclopentolate hydrochloride pharmaceutical preparations were purchased from Colircuci Ciclopejico Eye drops, 10 mg $mL^{-1}$ (Alcon cusi⁄S.A).
The tetrahydrozoline hydrochloride pharmaceutical preparations were purchased from Visine Eye drops 0.05%. (Pfizer Egypt S.A.E.)

**Solutions**
$4.4x10^{-3}$M of distigmine bromide, cyclopentolate hydrochloride, diaveridine hydrochloride and tetrahydrozoline hydrochloride was prepared in ethanol on using DDQ reagent. solutions were always freshly prepared by dissolving the accurate weighed amount in the proper amount of ethanol.
0.1%(w/v) of 2,3-dichloro-5,6-dicyano-p-benzoquinone (DDQ) reagent was prepared by dissolving the accurate weighed amount of 100 mg DDQ in 100 ml acetonitrile.
All solutions must be protected from light by keeping them in a dark coloured quickfit bottles during the whole work.
The water was always twice distilled from all glass equipments. Redistillation was carried out from alkaline permanganate solution.

**Equipments**
The spectrophotometric measurements were carried out using the manual spectronic 601 (Melton Roy Company), and Perkin Elmar automated spectrophotometer in the wavelength ranged from 200- 900 nm.

**Procedures**
Parameters affecting spectrophotometer determination of distigmine bromide, cyclopentolate hydrochloride, diaveridine hydrochloride and tetrahydrozoline hydrochloride via charge transfer complexation reaction with DDQ reagent:

**Selection of the suitable wavelength**
In calibrated 5 mL volumetric flask, different aliquots, containing 0.1-0.5 mL of 0.1%(w/v) of distigmine bromide, cyclopentolate hydrochloride, diaveridine hydrochloride and tetrahydrozoline hydrochloride were added to 0.1-0.5 mL of 0.1%(w/v) DDQ solution.
The volumes were completed to the mark with acetonitrile. The absorption spectra of the resulted CT complex products were scanned in the wavelength range λ = 350-900 nm from which the best wavelength for each drug was selected.

**Effect of time and temperature**
To select the optimum time and temperature for the complex formation, 0.1–0.5 mL of 0.1%(w/v) of distigmine bromide, cyclopentolate hydrochloride, diaveridine hydrochloride and tetrahydrozoline hydrochloride were added to 0.1-0.5 mL of 0.1%(w/v) of DDQ solution. The volumes were completed to the mark with the applicable solvent. First the absorbance was measured at different time intervals in the range 0–90 minutes in case of DDQ reagent. Second the absorbance was measured at different temperatures in the range from Zero–60 °C.

**Effect of DDQ concentration**
0.1-0.5 mL of 0.1% (w/v) of working solutions of distigmine bromide, cyclopentolate hydrochloride, diaveridine hydrochloride and tetrahydrozoline hydrochloride were added to different volumes of 0.1% (w/v) of DDQ solution. The volumes ranged from 0.1-2 mL and completed with acetonitrile to 5 ml. The absorbance was measured at the specific wavelength for each drug.

**Effect of organic solvents**
The same above procedure for drugs was followed using different organic solvents. Ethanol, chloroform, n-propanol, methanol, 1,4–dioxan, petroleum ether, 1,2-dichloroethane, acetonitrile and dimethyl formamide were tried to decide which of them causes more colour development.

**Stoichiometric ratio of the CT- complexes formed**
The stoichimetry of the charge transfer complexes formed was examined by applying continuous variation and molar ratio methods.

**(i) The continuous variation method**
A series of solutions were prepared by adding different volumes of 0.1% (w/v) of DDQ to $4.4x10^{-3}$ M of pharmaceutical drugs in case of DDQ reagent, so that the total number of

moles is kept constant. The procedures were followed as above and the absorbance data obtained were plotted against mole fraction of each drug.

**(ii) The molar ratio method**

1 mL of ($4.4x10^{-3}$ M) of pharmaceutical drugs were added to different volumes of 0.1% (w/v) of DDQ ranged from 0.1–3 mL in 5 mL volumetric flask and the absorbance was measured against ratio of reactants.

**Spectrophotometric determination of distigmine bromide, cyclopentolate hydrochloride, diaveridine hydrochloride and tetrahydrozoline hydrochloride with DDQ reagent**

**(i) Validity of Beer's law**

Suitable volumes of 0.1% (w/v) DDQ were added to different concentrations of 0.1% (w/v) of drugs (0.05-3 mg $mL^{-1}$) in case of DDQ procedure . The mixtures were completed up to 5 ml with acetonitrile. The absorbance of the coloured complex products were measured at the specific wavelengths against reagents blank prepared similarly without drugs.

**(ii) Between– day measurements**

In order to prove the validity and the applicability of the proposed method and the reproducibility of the results obtained, four replicates experiments at different concentrations of pharmaceutical drugs were carried out. Using the above mentioned procedures, the absorbance of the two samples were measured daily for four days and the results were recorded to make statistical calculations.

**Spectrophotometric determination of distigmine bromide, cyclopentolate hydrochloride, diaveridine hydrochloride and tetrahyrozoline hydrochloride in some pharmaceutical preparations**

Different concentrations of pharmaceutical drugs were added to suitable volumes of (0.1% w/v) DDQ reagent. The volumes were made up to the mark with acetonitrile in 5 mL calibrated measuring flask. The absorbance was measured at $\lambda_{max}$ = 460 nm for pharmaceutical drugs using DDQ reagents, against reagents blank.

**RESULTS AND DISCUSSION**

**Spectophotometric determination of distigmine bromide, cyclopentolate HCl, diaveridine HCl and tetrahydrozoline HCl via charge transfer complex formation:**

Molecular charge-transfer complexes (CT) are of particular interest in pharmaceutical science. They can be applied as useful means in the qualitative and quantitative analysis of different pharmaceutical compounds[18].

A charge transfer complex is the name that given to a stable molecular system formed in solution between an electron donating molecule, having sufficiently low ionization potential, and an electron accepting molecule having high electron affinity.

The principal feature of this type of complex formation is the appearance of a new and intense absorption bands in ultra-violet or visible region of spectrum. Absorption bands of this type are known as charge transfer bands, since they involve electronic transitions from orbital on the donor to the vacant orbital on the acceptor. Many explanations were given to the phenomenon based on quantum mechanical theory of Mulliken. The formation of molecular complexes from two aromatic molecules could arise from the transfer of an electron from a π–molecular orbital of the donor (Lewis base) to a vacant π–molecular orbital of the acceptor (Lewis acid) i.e. π-π* electronic interaction[19, 20].

Acceptors were classified, according to their general structure and type of substituents in the molecule. One of these classes was the substituted quinones, such as 7,7,8,8 tetracyanoquinodimethane (TCNQ), 2,3-dichloro-5,6-dicyano-p-benzoquinone(DDQ) and tetracyano ethylene (TCNE).

In the present investigation DDQ reagent is utilized as π-acceptor for the spectrophometric determination of distigmine bromide, cyclopentolate HCl, diaveridine HCl and tetrahydrozoline HCl .The main task of this study is to find fast, cheep, accurate and sensitive spectrophotometric method for the determination of distigmine bromide, cyclopentolate HCl, diaveridine HCl and tetrahydrozoline HCl in raw materials and in some commercial pharmaceutical preparations.

The method is based on the formation of CT complex between distigmine bromide, cyclopentolate HCl, diaveridine HCl and tetrahydrozoline HCl (electron donor) and DDQ reagent (electron acceptor). Different experimental conditions are carried out in order to select the optimum conditions suitable for CT complexes formation and hence quantitative determination of distigmine bromide, cyclopentolate HCl, diaveridine HCl and tetrahydrozoline HCl. Statistical treatment of the data obtained, like SD, RSD, Sandell sensitivity, ε, relative error, t- and F-tests are also made.

**Absorption spectra**

The absorption spectra of DDQ with distigmine bromide, cyclopentolate HCl, diaveridine HCl and tetrahydrozoline HCl in acetonitrile solvent (Fig. 1) showed the peak at λ = 460 nm was selected for distigmine bromide, cyclopentolate HCl, diaveridine HCl and tetrahydrozoline HCl-DDQ charge transfer complexes because it give the highest absorption intensity as indicated from the ε values. The polar solvents such as acetonitrile and methanol were reported[21,23] to promote complete transfer of electron from a donor (D) to the π- acceptor (A), [DDQ resulting in complete formation of DDQ radical anion ($A^{-}$) as a predominant chromogen.

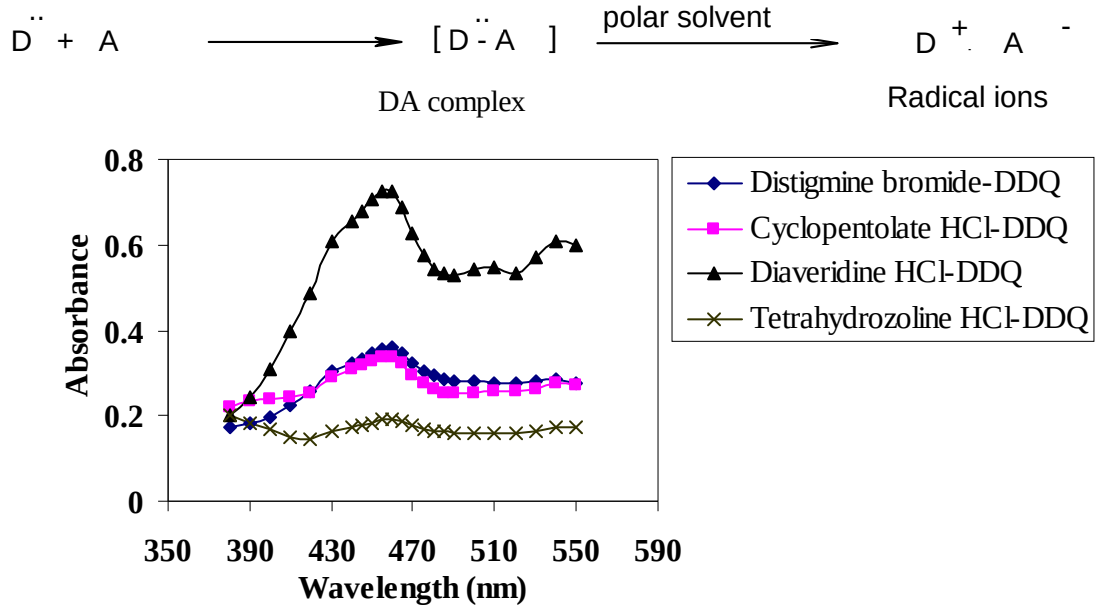



**Fig. 1: Absorption spectra of charge-transfer complexes of distigmine bromide, cyclopentolate HCl, diaveridine HCl and tetrahydrozoline HCl with DDQ in acetonitrile**

**Effect of solvent**

In order to select the suitable solvent for CT complex formation, the reaction of DDQ with distigmine bromide, cyclopentolate HCl, diaveridine HCl and tetrahydrozoline HCl is made in different solvents. These solvents included acetonitrile, chloroform, n-propanol, methanol, 1,4–dioxan, 1,2-dichloroethane, petroleum ether, ethanol and dimethyl formamide. The results obtained are shown in Figs. (2-5) and Table (1). From these results it is clear that acetonitrile is considered to be an ideal solvent for the colour reaction as it offers solvent capacity for DDQ and gives the highest yield of the radical as indicated by high ε values. This is because it possesses the high dielectric constant of all solvents examined; a property which is known to promote the dissociation of the original CT complex to radical ions i.e. the dissociation of donor–acceptor complex is promoted by the high ionizing power of the solvent.

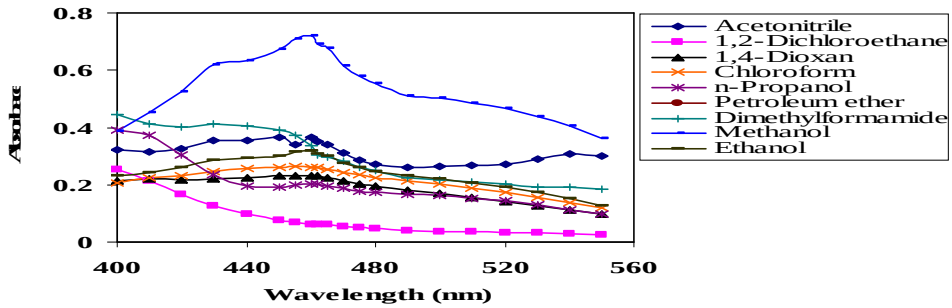



**Fig. 2: Effect of organic solvents on the absorption spectrum of distigmine bromide-DDQ-CT complex**

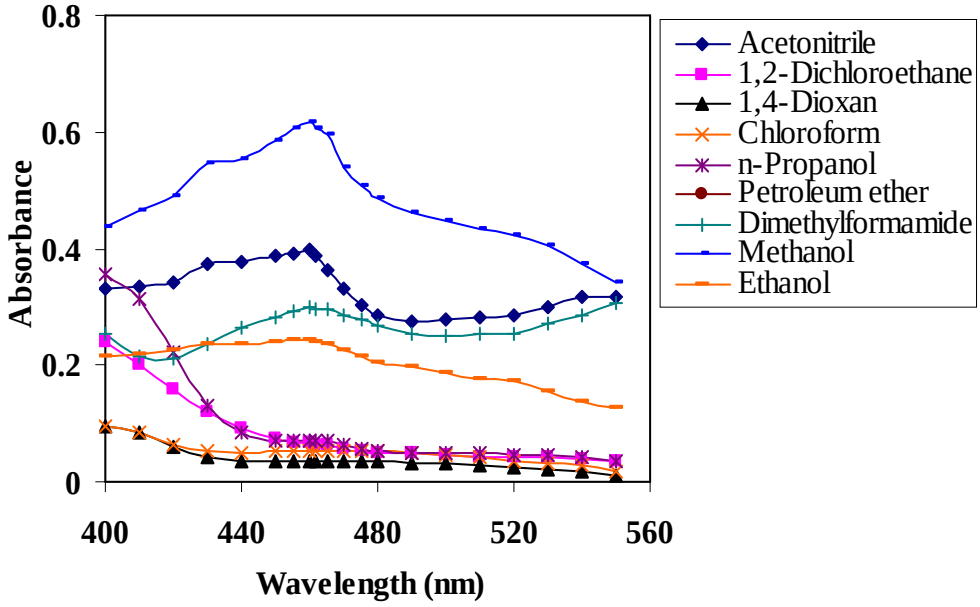



**Fig. 3: Effect of organic solvents on the absorption spectrum of cyclopentolate HCl-DDQ-CT complex**

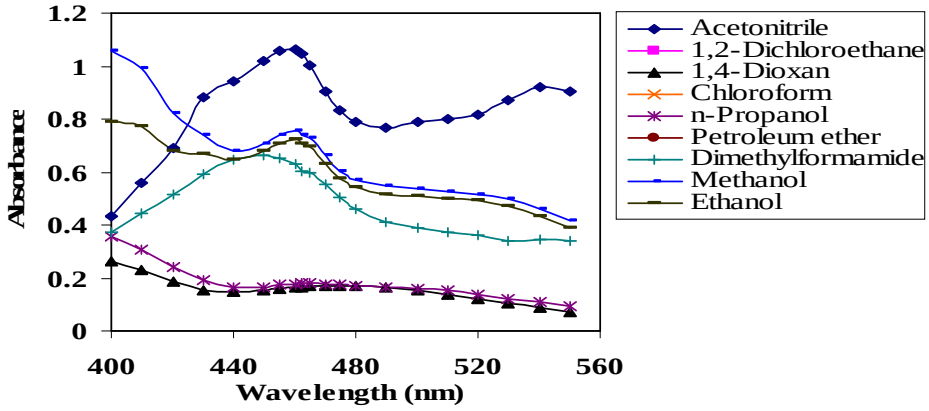



**Fig. 4: Effect of organic solvents on the absorption spectrum of diaveridine HCl-DDQ-CT complex**

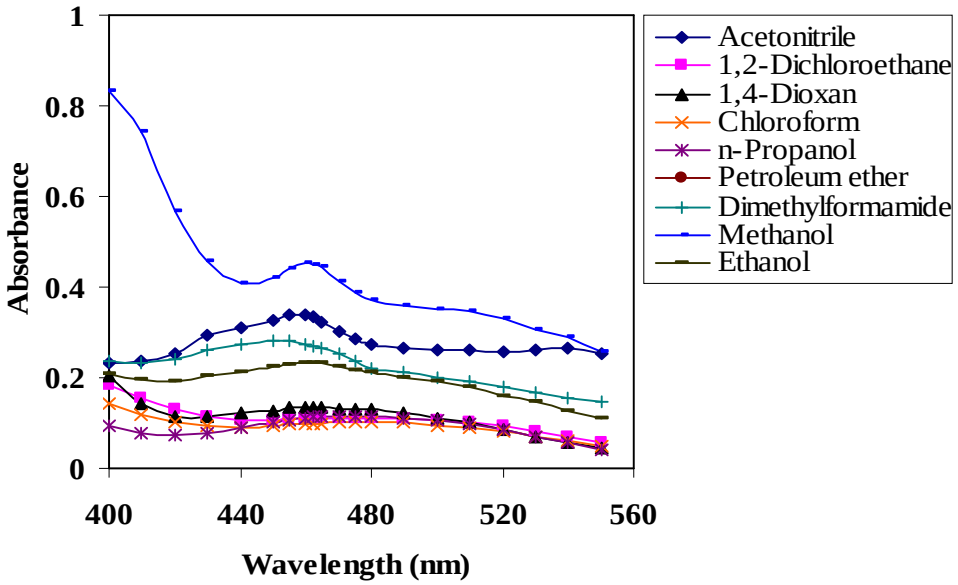



**Fig. 5: Effect of organic solvents on the absorption spectrum of tetrahydrozoline HCl-DDQ-CT complex**

**Table 1: The absorbance and molar absorptivity values of distigmine bromide, cyclopentolate HCl, diaveridine HCl and tetrahydrozoline HCl-DDQ-CT complexes in different solvents at $\lambda_{max.}$ = 460 nm**

| Solvent | Distigmine bromide | | Cyclopentolate HCl | | Diaveridine HCl | | Tetrahydrozoline HCl | |
|---|---|---|---|---|---|---|---|---|
| | A | ε(L.mol$^{-1}$.cm$^{-1}$) | A | ε(L.mol$^{-1}$.cm$^{-1}$) | A | ε(L.mol$^{-1}$.cm$^{-1}$) | A | ε(L.mol$^{-1}$.cm$^{-1}$) |
| Acetonitrile | 0.332 | 1.91×10$^3$ | 0.382 | 1.25×10$^3$ | 0.957 | 2.84×10$^3$ | 0.266 | 0.63×10$^3$ |
| Methanol | 0.643 | 3.71×10$^3$ | 0.599 | 1.96×10$^3$ | 0.651 | 1.93×10$^3$ | 0.386 | 0.92×10$^3$ |
| n-Propanol | 0.197 | 1.13×10$^3$ | 0.081 | 0.26×10$^3$ | 0.176 | 0.52×10$^3$ | 0.113 | 0.26×10$^3$ |
| Dimethylformamide | 0.191 | 1.11×10$^3$ | 0.251 | 0.83×10$^3$ | 0.208 | 0.62×10$^3$ | 0.155 | 0.37×10$^3$ |
| 1,4-Dioxan | 0.196 | 1.13×10$^3$ | 0.021 | 0.07×10$^3$ | 0.167 | 0.5×10$^3$ | 0.142 | 0.34×10$^3$ |
| Chloroform | 0.221 | 1.27×10$^3$ | 0.057 | 0.19×10$^3$ | ---- | ----- | 0.117 | 0.28×10$^3$ |
| Ethanol | 0.271 | 1.56×10$^3$ | 0.236 | 0.77×10$^3$ | 0.541 | 1.61×10$^3$ | 0.219 | 0.52×10$^3$ |
| 1,2-Dichloroethane | 0.078 | 0.44×10$^3$ | 0.077 | 0.26×10$^3$ | ---- | ----- | 0.122 | 0.29×10$^3$ |
| Petroleum ether | ---- | ------- | ---- | ------ | ---- | ----- | ---- | ----- |

**Effect of reagent concentration**

Figures (6) show the effect of 0.1 % (w/v) DDQ reagent on the quantitativeness of its reaction with distigmine bromide, bromide, cyclopentolate HCl, diaveridine HCl and tetrahydrozoline HCl, it is obvious that the drugs under study. It is also means that, maximum and reproducible colour intensities are obtained and higher concentration of reagent did not affect the colour intensity.

cyclopentolate HCl, diaveridine HCl and tetrahydrozoline HCl. It is found that, when various concentration of DDQ solution added to a constant concentration of distigmine (0.16-0.2) mg mL$^{-1}$ of DDQ solution is found to be sufficient for quantitative determination of

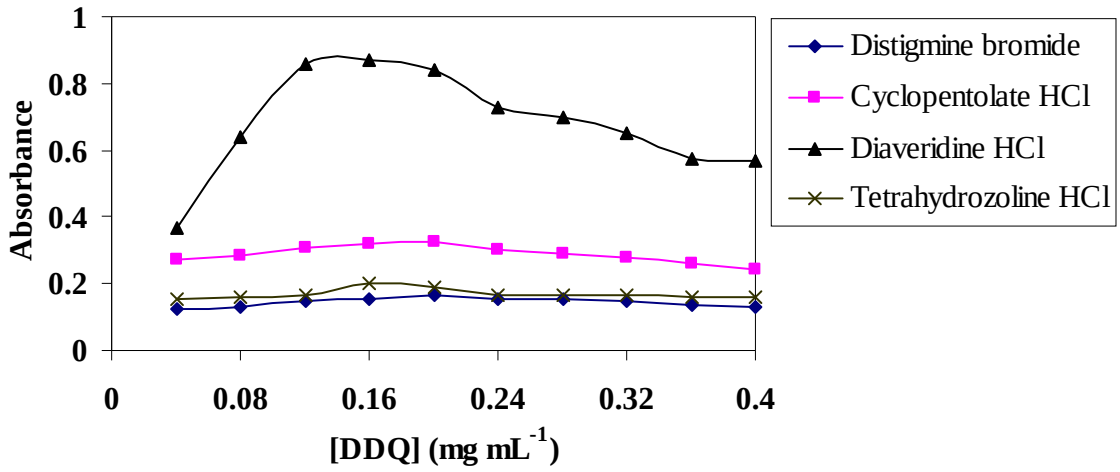



**Fig. 6: Effect of DDQ concentration on the formation of distigmine bromide, cyclopentolate HCl, diaveridine HCl and tetrahydrozoline HCl-CT complexes in acetonitrile**

**Effect of time**

Time of reaction has a pronounced effect on quantitativeness of the reaction between distigmine bromide, cyclopentolate HCl, diaveridine HCl and tetrahydrozoline HCl (electron donor) and DDQ reagent (electron acceptors). The optimum reaction time is determined spectrophotometrically at different temperatures and at λmax = 460 nm for DDQ reagents. Figure (7) show that complete colour development is attained after 30-50 minutes for DDQ reagent. Also the colour remains stable for one day at least using these reagents.

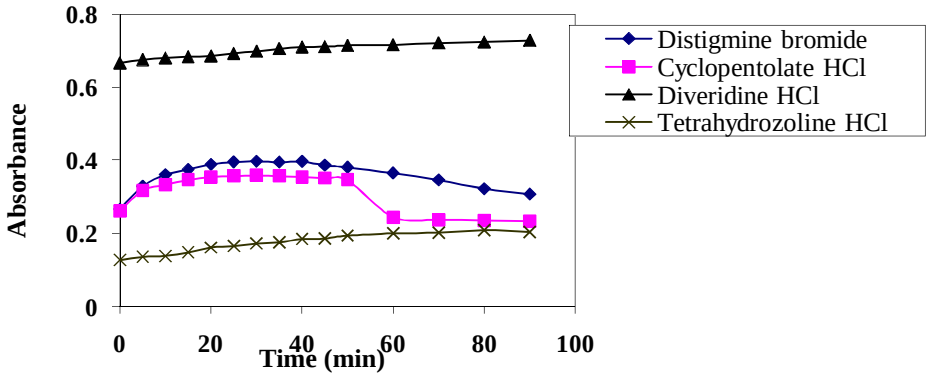



**Fig. 7: Effect of time on the absorbance of CT complexes of distigmine bromide, cyclopentolate HCl, diaveridine HCl and tetrahydrozoline HCl with DDQ in acetonitrile**

**Effect of temperature**

The aim of studying this factor using spectrophotometric method is to check the effect of temperature on the quantitativeness of these reactions. The effect of temperature in the range of 0 to 60 ºC on DDQ reactions with distigmine bromide, cyclopentolate HCl, diaveridine HCl and tetrahydrozoline HCl was studied. The absorbance of these CT complexes are measured at 460 nm for DDQ reagent against the blank solution prepared without the drug. The effect of temperature on these CT complexes is shown in Fig. (8). The given results show that the absorbance attains a maximum colour at temperature 25 ºC for DDQ reagent.

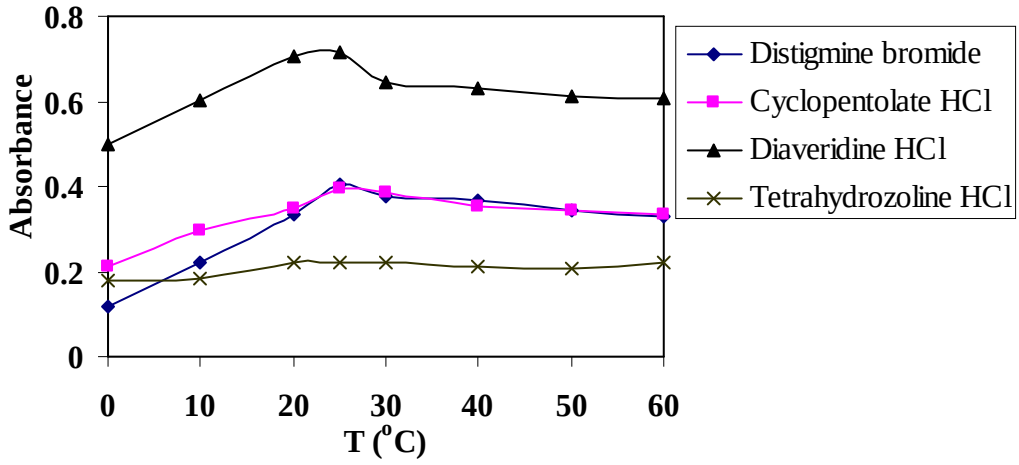



**Fig. 8: Effect of temperature (0-60 °C) on the absorbance of CT complexes of distigmine bromide, cyclopentolate HCl, diaveridine HCl and tetrahydrozoline HCl with DDQ in acetonitrile (t = 30-50 min, λ = 460 nm)**

**Stoichiometry of the CT complexes[24,25]**

Molar ratio and Job's continuous variation methods are applied in order to determine the suitable ratio between distigmine bromide, cyclopentolate HCl, diaveridine HCl and tetrahydrozoline HCl and DDQ reagent. Figs. (9-10) show that the interaction between these drugs and reagents occurs in equimolar basis, i.e the two straight lines are intersected at 1:1 [Drug]: [Reagents]. This means 1:1 complexes were formed between the drugs and DDQ reagent. The CT complexes formed between DDQ and distigmine bromide, cyclopentolate HCl, diaveridine HCl and tetrahydrozoline HCl takes place through the transfer of electron from a donor (drug) to the π-acceptor reagent (DDQ)[21, 22].

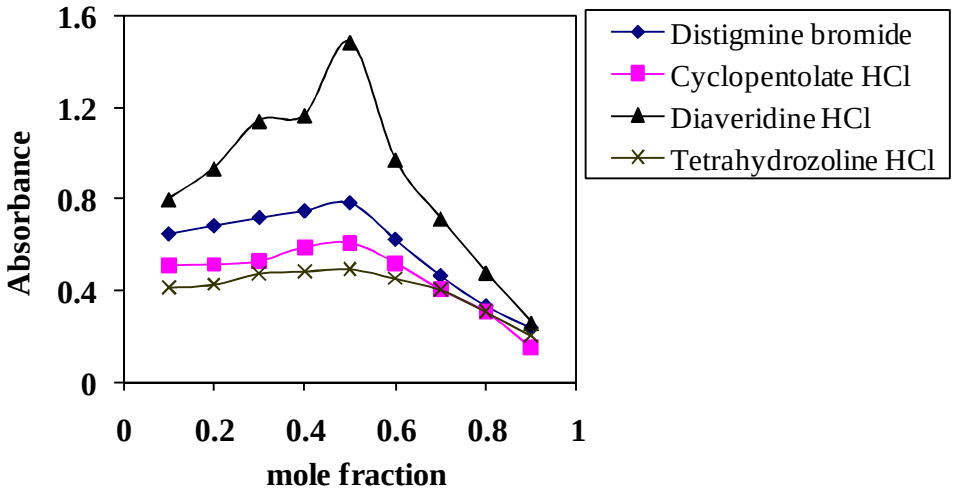



**Fig. 9: Job's method for distigmine bromide, cyclopentolate HCl, diaveridine HCl and tetrahydrozoline HCl-CT complexes with DDQ in acetonitrile**

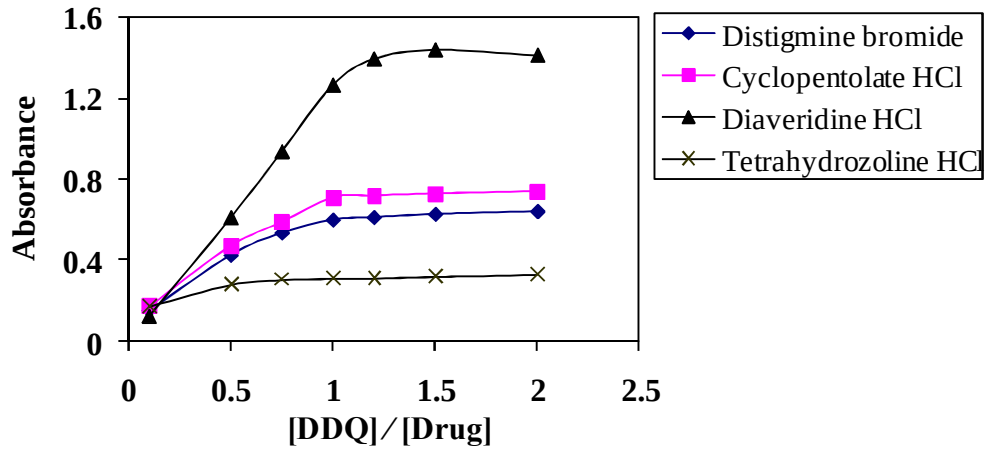
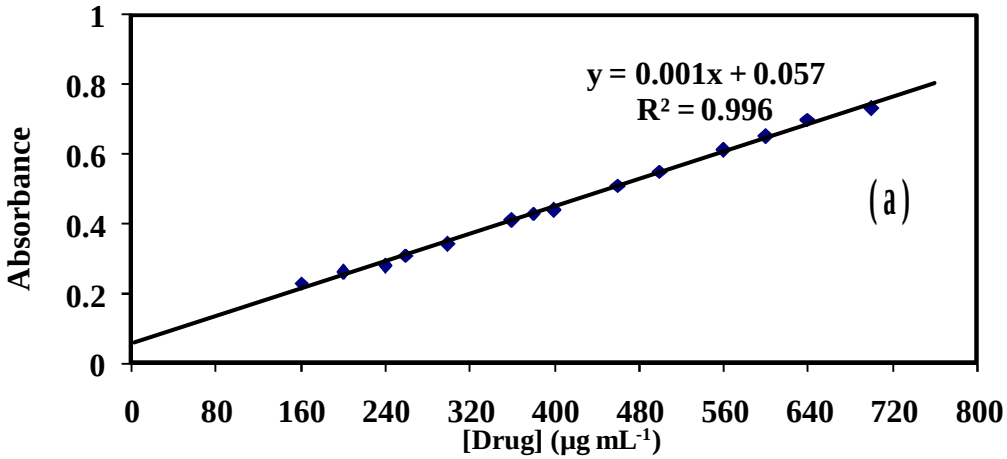



**Fig. 10: Molar ratio of distigmine bromide, cyclopentolate HCl, diaveridine HCl and tetrahydrozoline HCl-CT complexes with DDQ in acetonitrile**

**Obeyence to Beer's law**

After the selection of suitable pH, solvents, reagent concentrations, reaction time, temperature, and ratio it is also important to know the concentration limits of distigmine bromide, cyclopentolate HCl, diaveridine HCl and tetrahydrozoline HCl drugs at which these reactions are quantitative. Consequently, it is easy to apply this spectophotometric method to determine these drugs under investigation quantitatively in pharmaceutical formulations via its reaction with electron acceptor reagent like DDQ. Figs. (11-12) show the variation of absorbance with the change of the drug concentration. It is found that, Beer's law is valid over the concentration ranges from 160-700, 160-720, 5-130 and 20-640 µg $mL^{-1}$of distigmine bromide, cyclopentolate HCl, diaveridine HCl and tetrahydrozoline HCl using DDQ reagent, respectively.



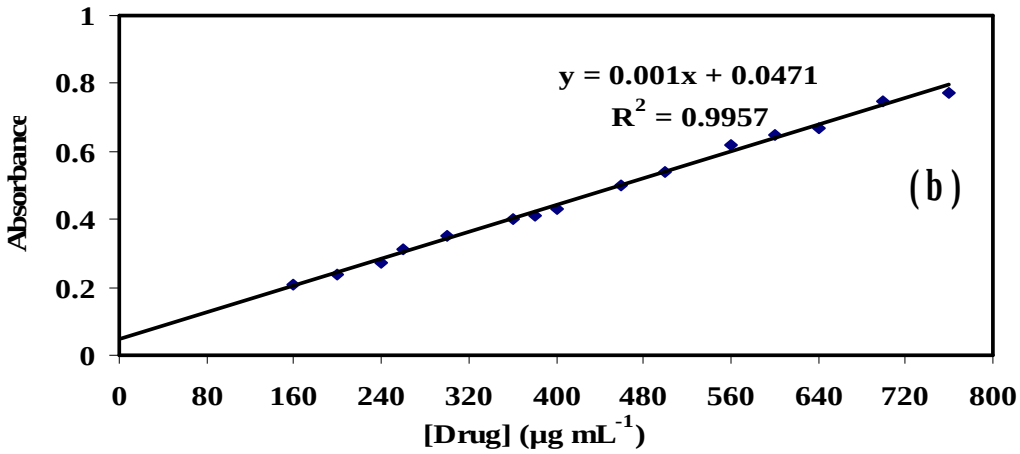



**Fig. 11: Obeyence of Beer's law of the CT reaction between DDQ and distigmine bromide (a) and cyclopentolate HCl (b) at selected optimum conditions**

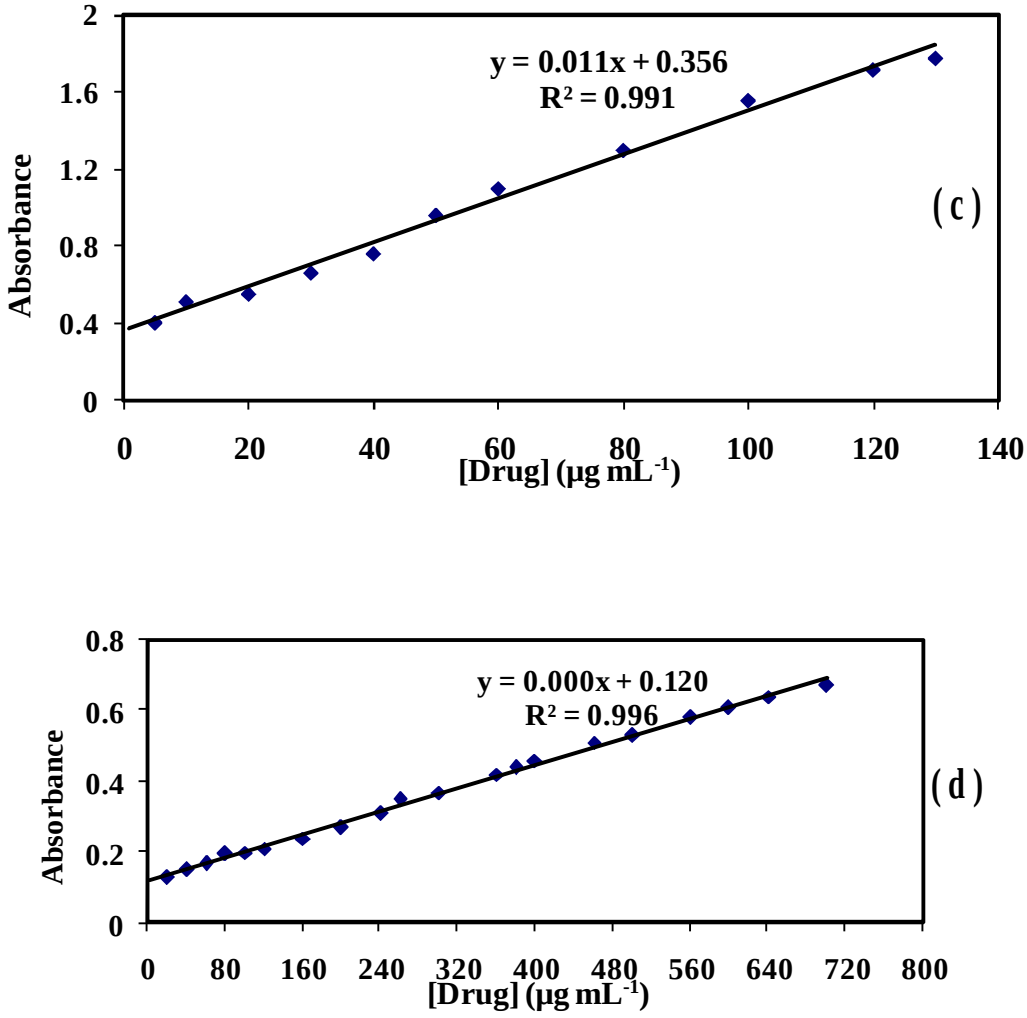



**Fig. 12: Obeyence of Beer's law of the CT reaction between DDQ and diaveridine HCl (c) and tetrahydrozoline HCl (d) at selected optimum conditions**

**Table 2: Spectral characteristics of DDQ-CT coloured reaction products and the analytical characteristics (accuracy and precision) of these reactions**

| Parameters | Results | | | |
|---|---|---|---|---|
| | distigmine bromide | cyclopentolate HCl | diaveridine HCl | tetrahydrozoline HCl |
| $\lambda_{max.}$, nm | 460 | 460 | 460 | 460 |
| Molar Absorptivity, L. $mol^{-1}.cm^{-1}$ | $1.91x10^3$ | $1.25x10^3$ | $2.37x10^3$ | $0.81x10^3$ |
| Sandell Sensitivity, µg $cm^{-2}$ | 0.91 | 2.44 | 1.70 | 5.20 |
| Beer's Law Limit, µg $mL^{-1}$ | 160 – 700 | 160 – 720 | 5 – 130 | 20 – 640 |
| Percentage Recovery, % | 99.86 – 100.25 | 99.58 – 100.8 | 99.5 - 101 | 99.68 – 100.4 |
| Range of Error, % | 0 – 0.25 | 0 – 0.77 | 0 -1.0 | 0.0 – 0.43 |
| Standard Deviation (SD) | 0.10 – 0.89 | 0.2 – 0.88 | 0.01 – 0.65 | 0.08 – 0.88 |
| Relative Standard Deviation, (RSD) % | 0.062 – 0.192 | 0.125 – 0.176 | 0.1 – 0.61 | 0.107 – 0.45 |
| Regression Equation*, Slope (b) | 0.001 | 0.001 | 0.0114 | 0.0008 |
| Intercept (a) | 0.0576 | 0.0471 | 0.3562 | 0.1205 |
| Correlation Coefficient (r ) | 0.9984 | 0.9978 | 0.9959 | 0.9983 |
| Detection limit, µg $mL^{-1}$ | 160 | 160 | 5 | 20 |

*A = a + bC; where C is the concentration in µg $mL^{-1}$

**Between-Day determination of distigmine bromide, cyclopentolate HCl, diaveridine HCl and tetrahydrozoline HCl.**

In order to prove the validity and applicability of the proposed method and reproducibility of the results obtained, four replicates experiments at four concentrations of distigmine bromide, cyclopentolate HCl, diaveridine HCl and tetrahydrozoline HCl were carried out. Table (3) show the values of the between-day relative standard deviations for different concentration of the drugs, obtained from experiments carried out over a period of four days. It is found that, the between day relative standard deviations are less than 1%, which indicates that the proposed method is highly reproducible and DDQ reagent is successfully applied to determine distigmine bromide, cyclopentolate HCl, diaveridine HCl and tetrahydrozoline HCl via the charge transfer reaction.

**Spectrophotometric determination of distigmine bromide, cyclopentolate HCl and tetrahydrozoline HCl in different pharmaceutical preparations.**

The spectrophotometric determination of distigmine bromide, cyclopentolate HCl and tetrahydrozoline HCl via their reaction with DDQ (strong electron acceptor) reagent are carried out. The results obtained are given in table (4). These data show that, the determined concentration of distigmine bromide, cyclopentolate HCl and tetrahydrozoline HCl drug by the proposed methods are closed to that calculated from the applied standard method. In order to check the confidence and correlation between the suggested spectrophotometric procedures and the official method[1,4,10] for determination of distigmine bromide, cyclopentolate HCl and tetrahydrozoline HCl, it is better to do the F- and t-tests for all the results (Table 4).

The calculated F- and t-tests at the 95 % confidence level do not exceed the theoretical values indicating non significant difference between the proposed and official method. The small values of SD and RSD indicate the reliability, accuracy and precision of the suggested procedures.

**Table 3: Between–day precision of the determination of distigmine bromide, cyclopentolate HCl, diaveridine HCl and tetrahydrozoline HCl using DDQ reagent**

| Drug | [Drug] Taken, µg mL$^{-1}$ | [Drug]* Found, µg mL$^{-1}$ | Percentage Recovery (%) | SD* | RSD* (%) |
|---|---|---|---|---|---|
| Distigmine bromide | 240.0 | 239.7 | 99.87 | 0.27 | 0.11 |
| | 300.0 | 299.7 | 99.90 | 0.39 | 0.13 |
| | 360.0 | 360.7 | 100.2 | 0.80 | 0.22 |
| | 400.0 | 400.1 | 100.0 | 0.80 | 0.20 |
| Cyclopentolate HCl | 240.0 | 239.8 | 99.90 | 0.43 | 0.18 |
| | 300.0 | 299.7 | 99.90 | 0.51 | 0.17 |
| | 360.0 | 359.6 | 99.90 | 0.44 | 0.12 |
| | 400.0 | 400.0 | 100.0 | 0.56 | 0.14 |
| Diaveridine HCl | 20.00 | 19.97 | 99.85 | 0.08 | 0.40 |
| | 40.00 | 39.96 | 99.90 | 0.14 | 0.35 |
| | 60.00 | 60.06 | 100.1 | 0.22 | 0.37 |
| | 80.00 | 80.10 | 100.1 | 0.24 | 0.30 |
| Tetrahydrozoline HCl | 100.0 | 99.95 | 99.95 | 0. 23 | 0.23 |
| | 160.0 | 159.9 | 99.98 | 0.19 | 0.12 |
| | 200.0 | 199.8 | 99.92 | 0.30 | 0.15 |
| | 260.0 | 260.0 | 100.0 | 0.42 | 0.16 |

* The average of four replicates.

**Table 4: Spectrophotometric determination of distigmine bromide, cyclopentolate HCl, diaveridine HCl and tetrahydrozoline HCl in different pharmaceutical preparations via their reactions with DDQ and official methods**

| Samples | Conc.Taken µg mL$^{-1}$ | [ Drug] µg mL$^{-1}$ | | SD* | SD** | F-test | t-test |
|---|---|---|---|---|---|---|---|
| | | DDQ method | Official method | | | | |
| $D_1$ | 300.0 | 300.01 | 299.95 | 0.058 | 0.095 | 2.68 | 2.31 |
| | 400.0 | 399.89 | 399.85 | 0.050 | 0.098 | 3.84 | 1.79 |
| $D_2$ | 260.0 | 259.98 | 260.01 | 0.070 | 0.106 | 2.30 | 0.96 |
| | 360.0 | 360.00 | 360.05 | 0.053 | 0.041 | 3.47 | 2.11 |
| $D_3$ | 100.0 | 100.00 | 99.97 | 0.044 | 0.085 | 3.73 | 1.52 |
| | 200.0 | 200.03 | 199.98 | 0.145 | 0.161 | 1.22 | 0.77 |

No. of replicates (n) = 5.
$D_1$ Ubreted tablets (5 mg/ tablet), Arab drug company, Egypt.
$D_2$ Colicuci Ciclopejico eye drops (10 mg mL$^{-1}$), Alcon cusi ⁄S.A.
$D_3$ Visine eye drops (0.05 %), Pfizer, Egypt, S.A.E.
* Standard deviation values using proposed method.
** Standard deviation values using official method.
Tabulated t – values at 95% confidence level = 2.77
Tabulated F – values at 95% confidence level = 6.39

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