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THE USE OF QUERCETIN FOR SPECTROPHOTOMETRIC DETERMINATION OF SOME CNS ACTING DRUGS

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تم فى هذا البحث إستحداث إثنين من الطرق الطيفية السهلة والبسيطة والدقيقة لتقدير سبعة عقارات للأدوية التى تؤثر على الجهاز العصبى والمركزى وهم هالوبيريدول والدروبيريدول وهيدروكلوريدات الكلوربرومازين والثيوريدازين والإيميبرامين والديزيبرامين والكلوميبرامين. وتعتمد الطريقة الأولى على تكوين أيون مركب بين الأدوية المدروسة والكوراستين المؤكسد فى محلول منظم للأس الهيدروجينى من ٤,٥ ٥ ، وقد تم إستخلاص الأيون المركب المتكون بواسطة مذيب الدايكلوروإثيان وقياس اللون الناتج عند طول موجى ٥٢٨ ٥٣٤ نانوميتر. وكانت هذه الطريقة تتبع قانون بيير عند تركيزات تتراوح بين ٢ ٤٥ ميكروجرام/مل. تعتمد الطريقة الثانية على قياس سرعة أكسدة الكوراستين إلى اللون الأحمر وقياسه عند طول موجى ٥١٥ نانوميتر ، وكذلك تأثير العقارات المدروسة على معدل الإنخفاض فى شدة إمتصاص اللون. وقد تمت الدراسة على الأدوية المذكورة عند تركيزات تتراوح من ٥ ٥٠ ميكروجرام/مل مستخدمين طرق الميل ، الوقت الثابت والوقت المتغير. وقد طبقت الطريقتين بنجاح على العقارات السابق ذكرها فى الصورة النقية ، كذلك على بعض الأشكال الصيدلية المحتوية على هذه العقارات ، وكانت النتائج مرضية وقد قورنت بنتائج التحليل الدستورية ، وكذلك الطرق المنشورة.

Two simple and sensitive spectrophotometric methods for the determination of seven CNS acting drugs namely; haloperidol, droperidol, chlorpromazine hydrochloride, thioridazine hydrochloride, imipramine hydrochloride, desipramine hydrochloride and clomipramine hydrochloride are presented. The first method is based on formation of an ion-pair complex between each of studied drugs and oxidized quercetin at pH range from 4.5 to 5.0. The colored complex formed was quantitatively extracted into dichloroethane, filtered over anhydrous sodium sulphate and then measured in the range of 528-534 nm. Beer's law was obeyed for all the studied drugs in the concentration range of 2-45 µg/ml. The second method is a rapid kinetic method based on in-situ oxidation of quercetin into a red colored compound measured at 515 nm. The rate of reaction between each of studied drugs and oxidized quercetin was followed by measuring the decrease in absorption intensity as a result of its reduction by each of the studied drugs. The studied compounds were determined in the concentration range of 5-50 µg/ml using slope, fixed time and variable time methods. The two methods were successfully applied to the analysis of studied drugs in the pure form and in commercial pharmaceuticals. The results were comparable with those obtained with either official or reported methods.

INTRODUCTION

The use of drugs with well demonestrated efficacy in psychiatric disorders has become wide spread since 1950s. Nowadays about 20% of prescriptions written in the United States are for medications intended for mental processes.[1]

Several analytical methods have been reported for determination of CNS acting drugs either in pure form or in their pharmaceutical preparations. These methods include; spectrophotometry,[2-22] spectrofluorimetry,[23-29] titrimetry,[30-35] chromatography,[36-48] flow injection analyses,[49-53] radio-immunoassay,[54-56] electrochemical methods[57-58] and chemiluminescence methods.[59-61]

In the present work oxidized quercetin is used for extractive spectrophotometric

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determination of seven CNS acting drugs which are listed in Table 1. In addition oxidized quercetin is used also for kinetic spectrophotometric determination of five CNS acting drug, namely, chlorpromazine HCl, thioridazine HCl, clomipramine HCl, imipramine HCl and desipramine HCl. In our laboratory oxidized quercetin has been reported for determination of drugs containing tertiary amine and quaternary ammonium salt moieties.[62] The use of quercetin as analytical reagent provides a fairly high extraction efficiency and sensitivity compared to other similar ion-pair reagents.

EXPERIMENTAL

Instrumentation

- A UV-1601, UV-Visible spectrophotometer (Shimadzu, Japan).
- pH-meter, model 3305 (Jenway, UK).

Materials

N- Bromosuccinimide (BDH, Poole, UK), quercetin (Merck, Darmstadt, Germany), haloperidol (%purity 99.75±0.55) and droperidol (%purity 99.21±0.43) (Gedeon Richter, Budapest, Hungary), imipramine HCl (%purity 100.26±0.36), clomipramine HCl (%purity 99.92±0.25) and desipramine HCl (%purity 99.28±0.57) (Novartis Pharma AG, Basle, Switzerland), chlorpromazine HCl (%purity 98.91±0.45) (May and Baker, England) and thioridazine HCl (%purity 98.35±0.60) (Swiss Pharma, Cairo).

Pharmaceutical preparations containing studied drugs were obtained from local market. Halodol deconoas® ampoules (B.N. 97B24/247, Janssen Pharmaceutica, Beerse, Belgium), safinace® tablets (1.5 mg B.N. 0010801, 5 mg B.N. 0010651, Kahira, Cairo, Egypt), droperidol and desipramine (laboratory prepared synthetic mixtures), neurazine® tablets (B.N. 143021, Misr, Cairo, Egypt), promacid® tablets (B.N. 15895, CID, Giza, Egypt), supranil® capsules (25 mg B.N. 01109127, 50 mg B.N. 91100194, ACAPI, Cairo, Egypt), melleril retard® tablets (200 mg B.N. 183, 30 mg B.N. 118) tofranil® tablets (B.N. 172) and anafranil® tablets (25 mg B.N.082, 75 mg B.N. 145) (Novartis Pharma AG, Basle, Switzerland).

- All other chemicals and solvents used in this work were of analytical grade.

Reagents and solutions

NBS solution

- Extractive spectrophotometric method.
  NBS solution 0.15 %w/v in distilled water and was prepared fresh daily.
- Kinetic spectrophotometric method:
  For phenothiazines 3.0×10^{-3} M solution
  For dibenzazepines 7.2×10^{-3} M solution.

Quercetin solution

- Extractive spectrophotometric method.
  Quercetin solution 0.1 %w/v in ethanol and was prepared fresh daily.
- Kinetic spectrophotometric method:
  For phenothiazines 3.0×10^{-3} M solution
  For dibenzazepines 7.2×10^{-3} M solution.

Table 1: Effect of different solvents on the absorption intensity and molar absorptivity of ion-pair complex of the studied drugs.

Solvent	Haloperidol**			Chloropromazine HCl**			Desipramine HCl**		
	λ_{max}	Abs.*	ε_{max}	λ_{max}	Abs.*	ε_{max}	λ_{max}	Abs.*	ε_{max}
Dichloroethane	530	0.671	10088.3	532	0.625	8883	532	0.643	7789.3
Chloroform	530	0.652	9817.7	532	0.614	8726.7	532	0.636	7704.5
Methylene chloride	530	0.653	9817.9	532	0.617	8769.3	532	0.637	7716.6
Carbon tetrachloride	290	0.038	511.3	295	0.042	596.9	305	0.049	593.6
Benzene	516	0.178	2676.2	515	0.190	2700.4	513	0.193	233.8

*Average of 4 determinations.
**Use 25 µg/ml from each drug.

Buffer solution

Teorell and Stenhagen buffer pH 2.0-12.0 was prepared.[63]

Standard drug solution

Standard drug solutions were prepared by dissolving 50 mg of the studied drug (as a salt) in 100 ml of distilled water except haloperidol and droperidol were prepared by dissolving 50 mg of the base in 100 ml methanol. Working standards covering the range 20-500 µg/ml were prepared by further dilutions.

Preparation of sample

Tablets and capsules

An accurately weighed amount of powder obtained from 20 tablets or capsules equivalent to 25 mg of the drug was transferred into 100 ml volumetric flask which contain about 50 ml distilled water (methanol in case of haloperidol and droperidol, then was shaked for 10 minutes, then was completed to 100 ml with distilled water (methanol in case of haloperidol and droperidol), was filtered and first portion of filterate was rejected. The prepared solution was diluted quantitatively to obtain the required concentration for assay.

Ampoules

The content of 10 ampoules was mixed well and an accurately measured volume of the solution equivalent to 50 mg of drug was quantitatively diluted with methanol to 100 ml. The resulting solution was diluted with methanol to obtain the required concentration for analysis.

General assay procedure

Extractive spectrophotometric method

One milliliter of standard drug solution was transferred into seperating funnel then 1 ml of buffer solution pH 5.0 was added. An aliquot of 1 ml of freshly prepared quercetin solution 0.1 %w/v was added followed by 1 ml of NBS solution 0.15 %w/v. The content was mixed well and shaked with two 10 ml portions of dichlorethane then the organic solvent extracts were combined. The organic solvent extract was filtered over 2 g anhydrous sodium sulfate then the absorbance was measured at specified wavelength listed in Table 2 against a blank treated similarly using 1 ml of drug solution solvent.

Kinetic spectrophotometric method

Two milliliters of quercetin solution was transferred into 50 ml volumetric flask followed by 8 ml of N-bromosuccinimide solution, then allowed to stand for 10 minutes till complete reaction between quercetin and NBS solution, a suitable aliquot of the studied drug solution was added to the contents of the flask. The solution is diluted to the mark with methanol. The solution and reagent blank were placed in spectrophotometer and absorbance change of the solution at 515 nm was recorded and apply any one from three kinetic methods.

1. Slope method

The variation of absorbance with time $\Delta A/\Delta t$ is plotted versus the concentration of the drug.

2. Fixed time method

The absorbance difference ΔA between 1 min. and 30 min. from initiation of reaction is plotted versus concentration of the drug.

3. Variable time method

The time required to decrease absorbance to 0.47 in case of phenothiazines or to 1.00 in case of dibenzazepines is measured for each concentration and inverse time 1/t is plotted versus concentration of the drug.

RESULTS AND DISCUSSION

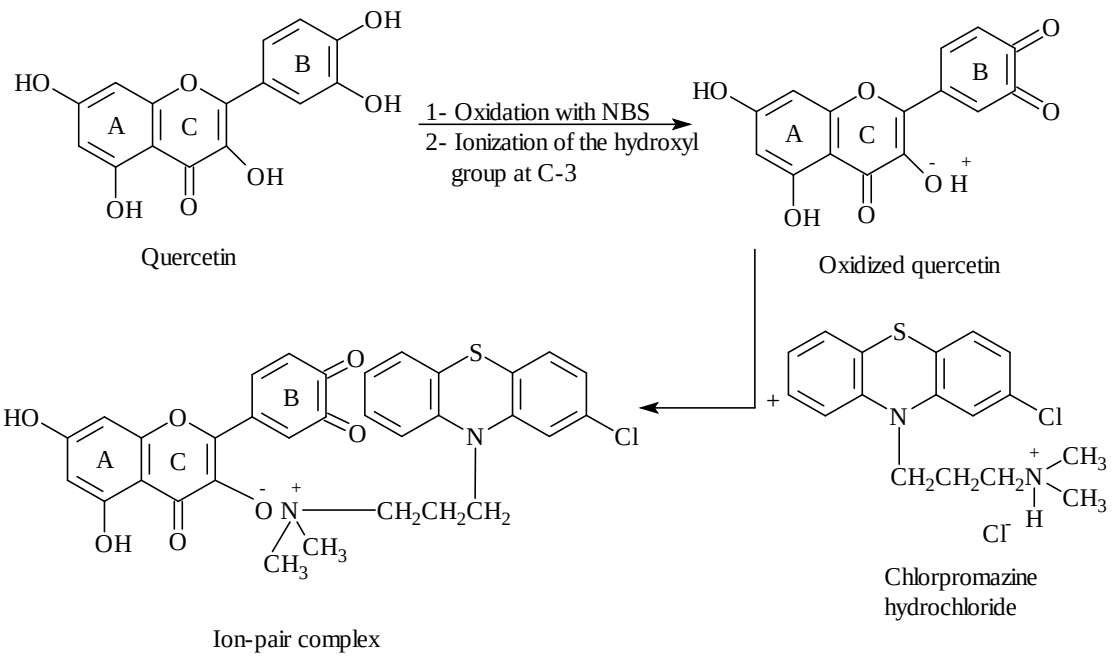
Extractive spectrophotometric method (Method A)

Ion-pair extraction method seems to be the most effective means for transferring the nitrogenous compounds from the aqueous phase to organic phase. The separated ion-pair complex could be determined colorimetrically. On the basis of the nature of quercetin and previous reports concerning its oxidation[64] a suggestion for the reaction mechanism is shown in Scheme 1. Quercetin being a flavanol undergoes oxidation of pyrocatechol moiety in ring B in presence of mild oxidants to produce highly colored o-quinone derivative. Ionization of hydroxyl group of ring C most probably become easier than the other two phenolic hydroxyl group in ring A. Leading to anion formation then the anion interacts with protonated tertiary amine from the drug (chlorpromazine hydrochloride) to form the color ion-pair complex.

Table 2: Quantitative parameters and statistical data for all the studied drugs using extractive spectrophotometric method.

	λ_{max} (nm)	ε_{max} (L $mol^{-1}cm^{-1}$)	Linearity range µg/ml	LOD	LOQ	Slope (SE)	Intercept (SE)	Correlation coefficient
1- Chlorpromazine hydrochloride	532	8613.0	2-45	0.796	2.654	0.02095 (0.00023)	0.05994 (0.00556)	0.9994
2- Thioridazine hydrochloride	528	9638.2	3-45	1.069	3.563	0.02046 (0.00029)	0.06647 (0.00734)	0.9991
3- Clomipramine hydrochloride	534	10482.8	3-50	1.137	3.791	0.02060 (0.000276)	0.07170 (0.00781)	0.9991
4- Imipramine hydrochloride	535	9950.0	2-45	1.014	3.380	0.02160 (0.000299)	0.0592 (0.00730)	0.9990
5- Desipramine hydrochloride	532	7813.5	2-50	1.091	3.638	0.02078 (0.000277)	0.07025 (0.00756)	0.9990
6- Haloperidol	530	9532.0	2-45	0.891	2.970	0.02370 (0.00030)	0.04847 (0.00704)	0.9991
7- Droperidol	534	9334.0	2-45	0.895	2.983	0.02068 (0.00025)	0.06623 (0.00617)	0.9992

LOD: Limit of detection µg/ml LOQ: Limit of quantitation µg/ml



Scheme 1

Absorption spectra

Quercetin is a yellow colored naturally occurring flavonoid (3,5,7,3',4'-pentahydroxy flavone). Upon oxidation using N-bromosuccinimide a red colored product (λ_{max}= 515 nm) was produced immediately. Figure 1 shows absorption spectrum of oxidized quercetin.

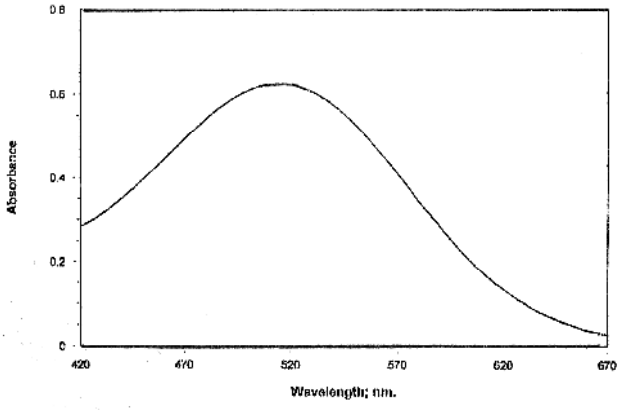


Fig. 1: Absorption spectrum of oxidized quercetin solution, $3x10^{-3}$ M.

The oxidized quercetin reacts with the drug at pH 5.0 to give highly colored ion-pair complex which is then extracted with dichloroethane and absorbance was measured at 530 nm.

Figure 2 shows absorption spectrum of the formed ion-pair complex between oxidized quercetin and 25 µg/ml chlorpromazine HCl.

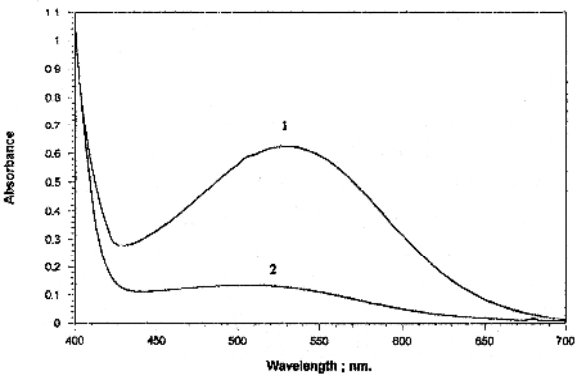


Fig. 2: Absorption spectra of an ion-pair complex of chlorpromazine HCl, 25 µg/ml **1** and a reagent blandk of oxidized quercetin **2**.

Optimization of variables for method A

Various parameters affecting complexation and extraction processes were investigated and optimized for all the studied drugs.

Effect of pH

Effect of pH was studied by extraction of colored complexes formed in the presence of buffer solution at different pH values. Figure 3 shows the effect of pH on ion-pair complexes

of studied drugs. It was found that maximum color intensity was obtained at pH 4.5-5.5 buffer solution of pH 5.0 was selected for subsequent work. At higher pH values the oxidized quercetin becomes unstable and the reddish-violet color in aqueous phase changes to yellow. At lower pH values reagent blank gives relatively high readings.

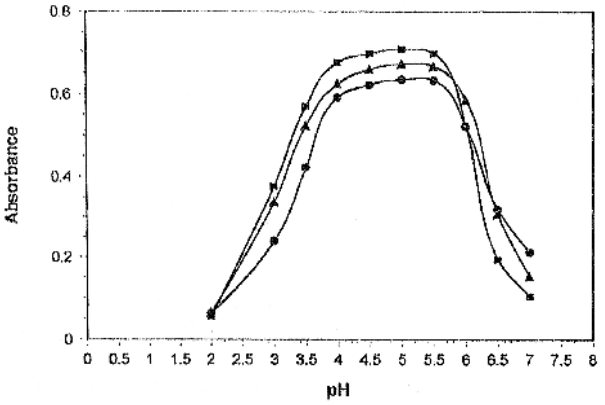


Fig. 3: Effect of pH on absorption intensity of the formed ion-pair complex using 25 µg/ml from ν Haloperidol, σ Chlorpromazine HCL, λ Desipramine HCl.

Effect of quercetin concentration

Several solutions of quercetin in the concentration range from 0.02 to 0.2 %w/v were prepared and 1 ml from each solution was used for general assay procedure. The obtained absorbances were plotted against quercetin concentration (Fig. 4). The higher color intensity was obtained when using quercetin at concentration range from 0.08 to 0.14 %w/v. Therefore 0.1% w/v quercetin concentration was selected for all subsequent work.

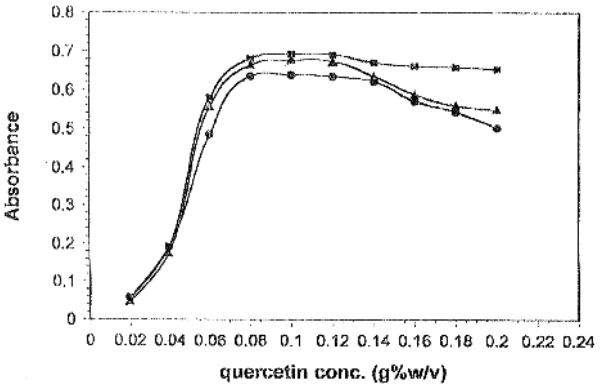


Fig. 4: Effect of quercetin concentration on absorption intensity of the formed ion-pair complex using 25 µg/ml from ν Haloperidol, σ Chlorpromazine HCL, λ Desipramine HCl.

Effect of NBS concentration

Several concentrations of NBS solution in the concentration range 0.025 to 0.25 %w/v were prepared and 1 ml of each concentration was added to quercetin solution and procedure was completed as in general procedure. Figure 5 shows the effect of NBS concentration on absorption intensity. It shows that 0.15 %w/v is the most suitable concentration for the determination of studied drugs. Concentrations greater than 0.175 %w/v showed a marked decrease in absorption intensities which may be due to the further oxidation of quercetin to other products.

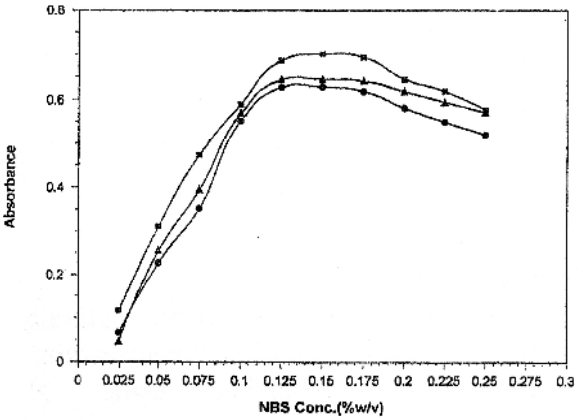


Fig. 5: Effect of NBS concentration on absorption intensity of the formed ion-pair complex using 25 µg/ml from ν Haloperidol, σ Chlorpromazine HCL, λ Desipramine HCl.

Effect of extraction solvent

The formed ion-pair complex was extracted with different organic solvents such as 1,2-dichloroethane, methylene chloride, chloroform, benzene and carbon tetrachloride. Table 1 shows that the highest absorbances were obtained using dichloroethane as extraction solvent. Chloroform and methylene chloride could be used. Highly non-polar solvents such as carbon tetrachloride were found to have very poor extraction efficiency.

Order of addition

All possible sequences of addition of the reactants and buffer solutions were carried out. A significant decrease in the absorption intensity was noticed when both the drug and the oxidant NBS were added to each other and followed by addition of quercetin. This may be attributed to oxidation of the drug with NBS to

give a compound which either does not react with oxidized quercetin or the formed ion-pair of this compound is poorly extracted into the organic phase. The most proper order was drug, buffer, quercetin and finally NBS.

Effect of reaction and stability time

Oxidation of quercetin by NBS was found to be rapid indicated by formation of reddish violet color in the aqueous phase. Figure 6 indicates the effect of reaction time on the absorption intensity of the formed ion-pair complexes of some studied drugs. It was found that the reaction between oxidized quercetin and the drug is very rapid (less than one min.). However after about 8 min. reaction time a gradual decrease in the absorption intensity was observed. Regarding stability time of the colored products in dichloroethane it was noticed that the absorbances were slightly increased in the first 5 min. and then remain stable for more than 1 hour. Results are shown in Figure 7.

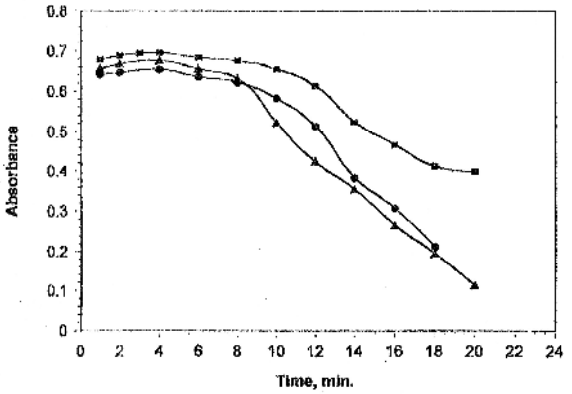


Fig. 6: Effect of reaction time on absorption intensity of the formed ion-pair complex using 25 µg/ml from ν Haloperidol, σ Chlorpromazine HCL, λ Desipramine HCl.

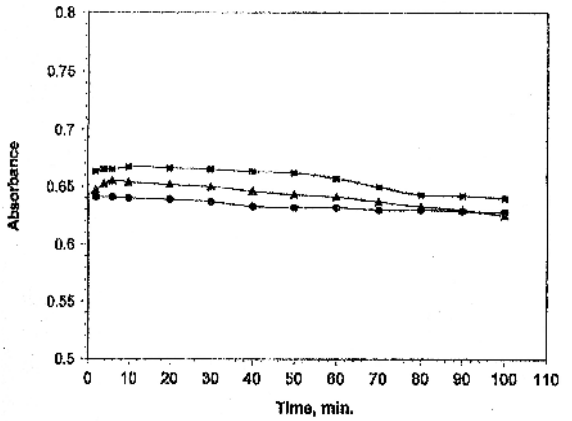


Fig. 7: Stability of the formed ion-pair complex using 25 µg/ml from ν Haloperidol, σ Chlorpromazine HCL, λ Desipramine HCl.

Analytical parameters

Table 2 shows quantitiave parameters and statistical data for all the studied drugs. Slope (b) was used as a measure of sensitivity of the proposed method. Intercept (a) was used as a measure of interferring back ground. Five replicate determinations at different concentration levels were carried out to test precision of the method. The RSD was found to be less than 1.5 % indicating reasonable repeatability of selected method. Regression analysis indicates excellent conformity with Beer's law over the concentration ranges listed in Table 2 for each drug.

Interference study

The effects of the presence of common tablet excepients such as; starch, sucrose, glucose, gum acacia, lactose, Mg-stearate and talc are studied. Results are listed in Table 3. Haloperidol was taken as a representative example. The results indicate that there is no interference from the frequently encountered excepients and additives. This is evidenced by the fact that all these additives are neutral and almost non extractable by organic solvents thus they do not contribute in the reaction at all. Also, the presence of traces of water in the organic solvent extracts may cause discrepancy in absorbance measurements and subsequent variation in results and so they must be removed by filteration over anhydrous sodium sulfate.

Table 3: Analysis of haloperidol[@] as a representative example in the presence of some tablet excepients using method A.

Ingredient	Amount added	% Recovery[*] ± S.D.
1- Starch	50 mg	99.50 ± 1.21
2- Sucrose	50 mg	99.58 ± 1.38
3- Glucose	50 mg	99.4 ± 1.25
4- Gum acacia	10 mg	98.7 ± 0.92
5- Lactose	10 mg	98.4 ± 1.11
6- Mg-stearate	10 mg	99.1 ± 1.08
7- Talc	10 mg	98.7 ± 1.3

[*]Average of five determinations.
[@]Use 25 µg/ml of haloperidol

Kinetic Spectrophotometric method (Method B)

Phenothiazines and diabenzazepines are able to reduce oxidized quercetin and decolorize it. Therefore, the rate of the reaction was determined through measurement of the decrease in absorbance at 515 nm. (Figs. 8 and 9).

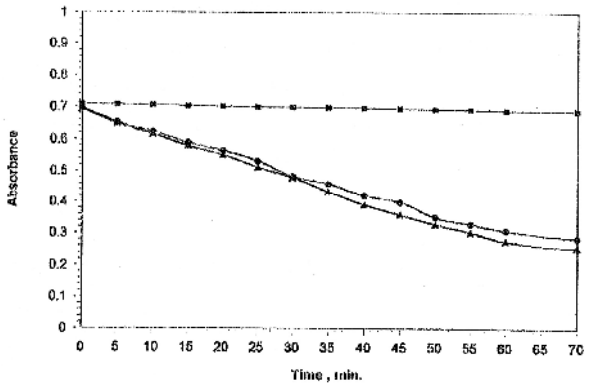


Fig. 8: Variation of absorbance of oxidized quercetin, $3x10^{-3}$ M with time upon addition of 30 µg/ml. ν Without drug, σ Chlorpromazine HCL, λ Thioridazine HCl.

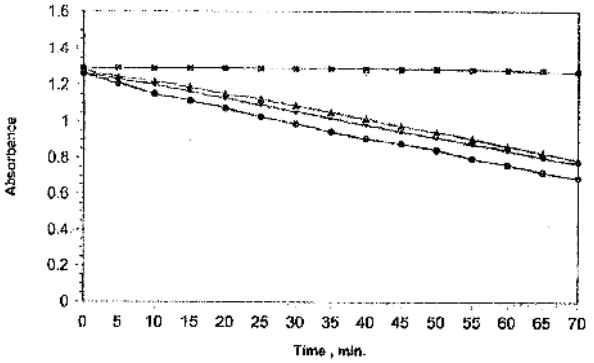


Fig. 9: Variation of absorbance of oxidized quercetin, $7.2x10^{-3}$ with time upon addition of 30 µg/ml. ι Clomipramine HCl, λ Desipramine HCl, σ Imipramine HCl, ν Without drug.

Optimization of variables for method B

Various parameters were investigated and optimized for all the studied drugs. All optimizations were carried out using fixed time kinetic method.

Effect of temperature

The temperature of 25° was chosen as optimum temperature to carry out the reaction as oxidized quercetin is unstable at higher temperatures.

Effect of oxidized quercetin concentration

The molar ratio for the reaction of quercetin and NBS was determined using continuous variation method and 1:4 ratio was obtained; respectively. Following this different molar concentrations from quercetin and NBS were prepared ($0.6\ x10^{-3}$ M – $10.8\ x10^{-3}$ M) keeping the ratio quercetin: NBS (1:4) then the procedure were completed as general procedure. The change in absorbance ΔA was recorded and plotted against concentration of oxidized quercetin (Fig. 10). It was found that optimum concentration of oxidized quercetin for phenothiazines (chlorpromazine HCl and thioridazine HCl) was $3.0x10^{-3}$ M and for dibenzazepine (clomipramine HCl, imipramine HCl and desipramine HCl) was 7.2×10^{-3} M.

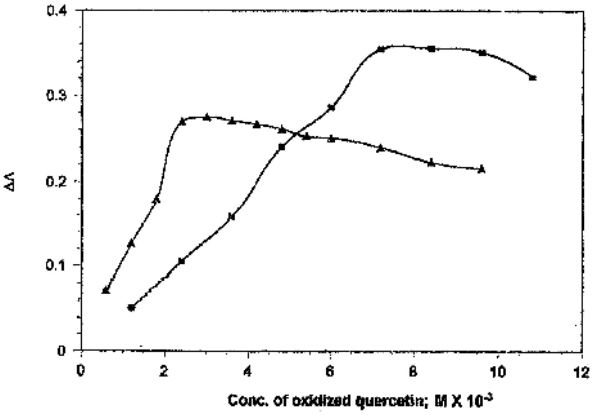


Fig. 10: Effect of oxidized quercetin concentration on absorption intensity difference caused by 30 µg/ml of ν Desipramine HCl, σ Chlorpromazine HCl.

Effect of dilution solvents

Dilution of reaction mixture (quercetin, NBS and drug) with any of solvents listed in Table 4 showed slight effect on λmax while the absorbance was affected. It was found that the highest absorption intensity was produced with methanol, therefore, it was used throughout this work.

Effect of addition of acid or alkali

To study effect of addition of acid or alkali one milliliter of HCl or NaOH solution range from 0.01 M to 0.05 M solution was added to reaction mixture. It was observed that the absorbance of oxidized quercetin decreased upon addition of acid or alkali. This attributed to that oxidized quercetin is unstable in acid or alkali.

Table 4: Effect of dilution solvent on absorbance of oxidized quercetin after addition of studied drug.

Solvent	Abs.*	λ_{max}*	Abs.**	λ_{max}**
1- Methanol	0.695	515	1.298	515
2- Ethanol	0.632	515	1.259	515
3- Water	0.712	530	1.344	530
4- Isopropyl alc.	0.601	515	1.211	515
5- Acetonitrile	0.565	515	1.091	515
6- 1,4-dioxan	0.418	500	0.958	500

*Using 30 µg/ml chlorpromazine HCl and $3x10^{-3}$ M oxidized quercetin.
**Using 30 µg/ml desipramine HCl and $7.2x10^{-3}$ M oxidized quercetin.

Kinetic data

- Slope method

The method depends on plotting the reaction rate $\Delta A/\Delta t$ versus concentration of the studied drug.

- Fixed time method

The method depends on plotting the absorbance difference between absorbance after 1 min. from beginning of reaction and absorbance after 30 min. $\Delta A = A_1 = A_{30}$ versus drug conceneration. This method was used for application on pharmaceutical preparations.

- Variable time method

The method depends on plotting 1/t reverse time required for absorbance to reach 0.47 for phenothiazines or 1.00 for dibenzazepines versus drug concentration. Table 5 shows the analytical data for determinaton of the studied drugs using different kinetic methods.

Interference study

Table 6 indicates that common tablet excepients did not interfere with the determination of the studied drugs. Among tablet excepients used in this study starch, sucrose, glucose, gum acacia, lactose, Mg-stearate and talc. Interference study of these common tablet excepients on the determination of chlorpromazine HCl using fixed time kinetic method shows good recoveries indicating no interference.

Analysis of pharmaceutical preparations

Some commercial dosage forms of the studied drugs were successfully analyzed by the proposed methods and official or reported methods. Method A was extractive spectrophotometric method and Method B was kinetic method (using fixed time method for applications). Recovery experiments were performed for each drug in its dosage forms. The results are listed in Table 7 for all the studied drugs.

Conclusion

The proposed methods are simple, rapid, sensitive and accurate methods for analysis of some CNS acting drugs. The first method involves formation of an ion-pair complex between the drug and oxidized quercetin while in the second method the drug reduces oxidized quercetin. The two methods could be applied for routine analysis of studied drugs in pharmaceutical preparations without interference from common tablet excepients.

Table 5: Quantitative parameters and statistical data for kinetic spectrophotometric method.

Drug	Linearity range (μg/ml)	Slope (SE)	Intercept (SE)	r	LOD	LOQ
1- Chlorpromazine HCl						
i-Slope method	5-50	0.000179 ($2.75x10^{-6}$)	0.002076 ($8.54x10^{-5}$)	0.9990	1.431	4.771
ii- Fixed time method	5-50	0.005183 ($7.75x10^{-5}$)	0.060667 (0.002403)	0.9991	1.391	4.636
iii- Variable time method	5-45	0.000903 ($2.13x10^{-5}$)	0.007781 (0.000599)	0.9981	1.990	6.633
2- Thioridazine HCl						
i-Slope method	5-50	0.000191 ($4.38x10^{-6}$)	0.001671 (0.000136)	0.9979	2.136	7.120
ii-Fixed time method	5-50	0.005545 (0.000119)	0.048200 (0.003678)	0.9981	1.990	6.633
iii-Variable time method	5-50	0.001109 ($2.53x10^{-5}$)	0.005883 (0.000786)	0.9979	2.126	7.087
3- Desipramine HCl						
i-Slope method	5-50	0.000304 ($6.53x10^{-6}$)	-0.000380 (0.000203)	0.9982	2.003	6.678
ii-Fixed time method	5-50	0.008817 (0.000190)	-0.00118 (0.005891)	0.9981	2.004	6.681
iii-Variable time method	5-45	0.001079 ($2.3x10^{-5}$)	0.003741 (0.000646)	0.9984	1.796	5.987
4-Clomipramine HCl						
i-Slope method	5-50	0.000295 ($5.7x10^{-6}$)	-0.00032 (0.000177)	0.9985	1.800	6.000
ii-Fixed time method	5-50	0.008577 (0.000149)	-0.010470 (0.004619)	0.9988	1.616	5.385
iii-Variable time method	5-50	0.000889 ($1.42x10^{-5}$)	0.005426 (0.000440)	0.9989	1.485	4.949
5- Imipramine HCl						
i-Slope method	5-50	0.000291 ($6.26x10^{-6}$)	-0.000350 (0.000194)	0.9982	2.000	6.667
ii-Fixed time method	5-50	0.008447 (0.000180)	-0.01000 (0.005579)	0.9982	1.981	6.605
iii-Variable time methods	5-50	0.001283 ($2.97x10^{-5}$)	0.001159 (0.000865)	0.9981	2.023	6.742

LOD : Limit of detection LOQ : Limit of quantitation

Table 6: Analysis of chlorpromazine HCl[@] in presence of some common additives using fixed time kinetic method.

Ingredient	Amount added	% Recovery[*] ± S.D.
1- Starch	100 mg	98.35 ± 0.91
2- Sucrose	100 mg	98.73 ± 1.16
3- Glucose	100 mg	97.85 ± 1.06
4- Gum acacia	20 mg	98.48 ± 0.82
5- Lactose	20 mg	98.35 ± 1.05
6- Mg-stearate	10 mg	97.7 ± 1.44
7- Talc	20 mg	98.22 ± 1.16

[*]Average of four determinations [@]using 30 μg/ml chlorpromazine HCl

Table 7: Determination of studied drugs in some pharmaceutical preparations using proposed and official methods.

Product	Ingredient (content, mg)	% Recovery* ± SD		
		Method A	Method B	Official method
1- Halodol deconas (ampoules)[a]	Haloperidol (50 mg)	99.16±1.05 **t= 1.6700, F= 1.8420	-----	100.14±0.77
2- Safinace (tablets)[a]	Haloperidol (5 mg)	99.01±0.92 t= 1.5376, F= 1.1146	-----	99.88±0.87
3- Safinace (tablets)[a]	Haloperidol (1.5 mg)	98.98±1.00 t= 2.219, F= 1.6216	-----	100.24±0.78
4- Droperidol (synthetic mix.)***[c]	Droperidol (5 mg)	99.01±0.92 t= 0.032, F= 1.0064	-----	99.40±1.10
5- Promacid (tablets)[b]	Chlorpromazine HCl (100 mg)	99.09±1.02 t= 0.492, F= 1.3796	98.78±1.56 t= 0.7617, F= 3.196	99.38±0.87
6- Neurazine (tablets)[b]	Chlorpromazine HCl (100 mg)	99.12±1.13 t= 0.128, F= 1.639	98.88±1.55 t=0.4066, F=3.075	99.20±0.88
7- Melleril retard (tablets)[b]	Thioridazine HCl (30 mg)	99.01±1.20 t= 0.796, F= 2.535	98.64±1.59 t= 1.1140, F= 4.430	99.56±0.76
8- Melleril retard (tablets)[b]	Thioridazine HCl (200 mg)	99.15±1.16 t= 0.234, F= 1.797	98.64±1.54 t= 0.8260, F= 3.150	99.30±0.86
8- Desipramine (synthetic mix.)***[b]	Desipramine HCl (25 mg)	99.28±1.10 t= 0.893, F= 1.760	98.77±1.72 t= 1.249, F= 4.200	99.84±0.84
10- Tofranil (tablets)[b]	Imipramine HCl (25 mg)	99.60±0.91 t= 0.262, F= 1.152	99.78±1.86 t= 0.357, F= 4.820	99.46±0.85
11- Supranil (capsules)[b]	Clomipramine HCl (50 mg)	98.94±0.94 t= 0.626, F= 1.319	98.28±1.70 t= 1.193, F= 4.320	99.30±0.82
12- Supranil (capsules)[b]	Clomipramine HCl (25 mg)	99.13±1.03 t= 0.969, F= 1.163	100.70±1.45 t= 1.234, F= 2.280	99.74±0.96
13- Anafranil SR (tablets)[b]	Clomipramine HCl (75 mg)	99.69±1.045 t= 0.533, F= 1.274	99.50±1.71 t= 0.244, F= 4.580	99.40±0.89
14- Anafranil (tablets)[b]	Clomipramine HCl (25 mg)	99.16±0.99 t= 0.398, F= 1.231	99.90±1.30 t= 0.720, F= 2.160	99.29±0.80

Method A Extractive spectrophotometric method
Method B Fixed time kinetic method
*Average of five determinations
**Theoritical values at 95% confidence limit (t= 2.306, F= 6.388)
***Laboratory prepared synthetic mixture
[a]According to USP 1990 [b]According to BP 1998 [c]According to Ref. [8]

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