Univariate and Simplex Optimization for The Analysis of Diphenhydramine-HCl Through Ion-pair Formation

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Abstract

A Simple, rapid and sensitive extractive and spectrophotometric method has been described for the analysis of diphenhyldramine –HCl (DPH) in pure form and in pharmaceutical formulations. The method is based on the formation of chloroform soluble ion-pair complex with Bromophenol blue(BPB) in a phthalate buffer at pH 3.0.The extracted complex shows maximum absorbance at 410 nm. Beer's law is obeyed in the concentration range 0.2-25.0 $\mu g.ml^{-1}$. The molar absorptivity and Sandell's sensitivity for the system being $2.416x10^4$ $L.mol^{-1}.cm^{-1}$ and 0.012$\mu g.cm^{-2}$, respectively. The limit of detection was found to be 0.155 $\mu g.ml^{-1}$.

The proposed method was successfully applied to the determination of the drug in pharmaceutical formulation and satisfactory results were obtained.

Key words: The Analysis of Diphenhydramine-HCl Through Ion-pair Formation, Univariate and Simplex Optimization

Introduction

Diphenhydramine, is chemically known as 2-(diphinylmethoxy)-N-N-dimethylamine hydrochloride (DPH), it is an antihistaminic species in the H_1-receptor antagonist that can be used as antiallergic, antiemetic and antitussive drugs found in many pharmaceutical preparation.[1].

Several analytical methods were previously proposed for determining DPH in pharmaceutical samples including titrimetry[2], fluorimetry[3], HPLC[4], HPTLC[5], capillary electrophoresis[6], gas chromatography[7] and electrochemical analysis[8].

UV-visible spectrophotometry is still considered to be the most convenient method for the assay of different classes of drugs in pure, pharmaceutical formulation and in biological samples, because of its simplicity and reasonable sensitivity with significant economical, advantages. Several spectrophotometric methods have been reported for the determination of DPH in bulk material and dosage forms. San Andre's [9] have described a spectrophotometric method based on measuring the critical micelle concentration of mixed sodium dodecyl sulfate-antihistamine aggregates. In another extractive spectrophotometrice method [10] the drug was precipitated with reineckate and the ion-pair complex was extracted with nitrobenzene and measurement at 520nm. The drug has also been determined

spectrophotometrically via the formation of an-ion-pair complex with Bromocresol-Green at pH 3.0 [11]. On the other hand, Bromophenol blue which is an anionic dye has been used as a precipitating reagent to form an ion-pair complex as well as in other flow-injection procedures with a turbimetric determination [12], here, the reaction between DPH and Bromophenol blue takes place on-line and no liquid extraction is required.

In experimental chemistry, the optimization of technical system is the process of the adjusting the control variables to find the levels that achieve the best optimization .Usually, many conflicting responses must be optimized simultaneously. In lack of systematic approaches the optimization is done by trial and error, or by changing one control variable at a time while holding the rest constant, such methods require a lot of experiments to be carried out. Simplex optimization of experimental parameters was first introduced by spindly [13],and then modified by Nedler [14]and Albery [15]. A simplex is a geometric figure in which there are n+1 vertices, where (n) represents the number of variables [16], the method found a lot of applications in field of analytical chemistry [17,18], because it offers the capability of optimizing several factors simultaneously depending on a statistical design search to find the maxima or minima of response, by rejecting the point producing the worst response and a replacement of it by the new point which is obtained statistically.

The present work describes the utility of Bromophenol blue (BPB) reagent for spectrophotometric determination of Diphenhydramine-HCL in pure form as well as in the dosage form. In addition, the optimizations of chemical dependent variables of affecting absorbance have been studied by using modified simplex method (MSM) via computer program.

Experimental

Apparatus

A Cintra 5 GBC.Scintific Equipment spectrophotometer with 1 cm quartz cells were used for absorbance measurements. pH-meter DW-9421 from Philips instrument, a Sartorius BL 210S balance, and a Pentium 4 computer (DELL) was used for data processing.

Material and Reagents

All of the used Chemicals were of analytical reagent grade unless otherwise is mentioned. Diphenhydramine - HCl was kindly provided by the State Company for Drug Industries and Medical Appliances, Samara-Iraq (SDI).

Bromophenol blue(BPB) (Aldrich), 0.1% (w/v) solution was prepared by dissolving 0.1 g of the dye in 5 ml of methanol and then the solution was diluted to a final volume of 100 ml with distilled water. Working solutions were freshly prepared by subsequent dilutions.

Hydrochloric acid (Aldrich), ~ 0.1 M , a 0.85 ml of concentrated hydrochloric acid (sp.gr. 1.18, 37%) was added to 50 ml distilled water and diluted to the mark in a 100 ml calibrated flask .

Sodium hydroxide (Fluka), ~ 0.1 M, was prepared by dissolving 0.40 g of sodium hydroxide in 25 ml distilled water and was diluted to 100 ml in volumetric flask with distilled water.

Phthalate buffer solution, 0.1M, was prepared by dissolving 4.08 g of potassium hydrogen phthalate (MERCK) in 25 ml distilled water and was diluted to 200 ml in volumetric flask with distilled water. The pH was adjusted to 3.0 by using few drops of 0.1M HCl and\or 0.1M NaOH {19].

Standard drugs solution, Diphenhydramine stock solution (250 $\mu g.ml^{-1}$), was prepared by dissolving 25 mg of the drug in 5ml distilled water and was diluted to 100 ml in a volumetric flask with distilled water . Working solutions were freshly prepared by subsequent dilutions.

General Procedure

Assay procedure for pure Diphenhydramine

1 ml aliquots of Diphenhydramine standard solution containing (1-125 µg) were transferred into a series of 50 ml separating funnels. To each funnel 2 ml of phthalate buffer of pH 3.0 and 1 ml of 0.05% BPB reagent solutions were added. The separating funnels were shaken with 5 ml chloroform for one minute. The two phases were then allowed for clear separation and the absorbance of the yellow colored organic phase was measured at 410 nm against a reagent blank prepared similarly without addition of Diphenhydramine. The calibration graph was constructed by plotting the measured absorbance of the organic phase against the drug concentration.

Analysis of pharmaceutical formulation

i. In tablets:

Ten tablets were weighed and grounded into a fine powder. An amount equivalent to 25mg of the cited drug was weighed accurately and transferred into 25ml beaker and then was dissolved in 5ml of distilled water and was diluted to 100ml in a volumetric flask. The resulted solution was then filtered through a Whatman filter paper No. 41 to avoid any suspended material before use. Working solutions were freshly prepared by subsequent dilution with distilled water and analyzed by the recommended procedure.

ii. In syrup :

An accurately volume of the mixed Allermine syrup equivalent to 100mg of drug base was quantitatively transferred into a 100ml standard flask and diluted up to the mark with distilled water. An aliquot of the solution was transferred into a separating funnel and treated as described for tablets.

Results and Discussion

Extractive spectrophotometric procedures are popular for their sensitivity in the assay of drugs and hence, ion pair extractive spectrophotometry has received considerable attention for the quantitative determination of many pharmaceutical compounds [20, 21].

Preliminary investigations revealed that DPH reacted with BPB in acidic buffer to yield chloroform-soluble ion-pair complex exhibiting an absorption maximum at 410 nm against the reagent blank (Fig1). Under experimental conditions the corresponding reagent blank showed a negligible absorbance.

Optimization of experimental variables:-

Univariable method:

The experimental variables affecting the development and stability of the ion-pair complex were achieved through a number of preliminary experiments; these variables include pH, reaction time, reagent concentration, order of addition, shaking time and the type of organic solvent used for extraction. For this reason, a variable was modified while maintaining the other variables at their constant values, then by maintaining that variable at its optimized values, another was modified; all variable were optimized via this method.

Effect of pH:

In order to establish the optimum pH range, diphenhydramine-HCl solutions was mixed separately with specified volume of BPB. The pH was then adjusted to a value between (2.0-5.5) with few drops of or 0.1M NaOH or 0.1M HCl. It was noticed that maximum color intensity and constant absorbance was found at pH 3.0 (Fig 2). Low absorbance values were

observed in solution with higher or lower pH than the optimum value. Hence, a pH of 3.0 was used in all the subsequent experimental work.

Effect of reaction time:

The optimum reaction time was determined by following the color development at ambient temperature (25 ± 2 °C). It was found that the reaction was instantaneous hence, the product attained maximum and constant absorbance immediately after the diphenhydramine-HCl has been reacted with BPB and the color obtained remained strictly unaltered for 24hr.

Effect of reagent concentration:

The influence of excess reagent concentration on the absorbance of the complex is illustrated in (Fig 3). 0.05% solution of BPB was found to develop the color and reach its maximum intensity, after which no more increase in absorbance was obtained; therefore 0.05% solution was used.

Effect of shaking time:

The optimum shaking time for the complete extraction of the ion-pair complex with chloroform was studied for the period of 1-8 minutes (Table 1). It was found that the minimum shaking time for complete extraction was 1 minute at room temperature (i.e. 25 ±2°C).

Effect of the extracting solvent:

Several organic solvents, namely toluene, carbon tetrachloride, benzene, 1,2-dichloro ethane in addition to chloroform, were examined for their ability to extract the drug-dye ion-pair. The latter was found to be the most suitable solvent in terms of extraction efficiency (Table 2). On the other hand, it was observed that only a single extraction with 5ml portion of chloroform was adequate to achieve a quantitative recovery of the complex.

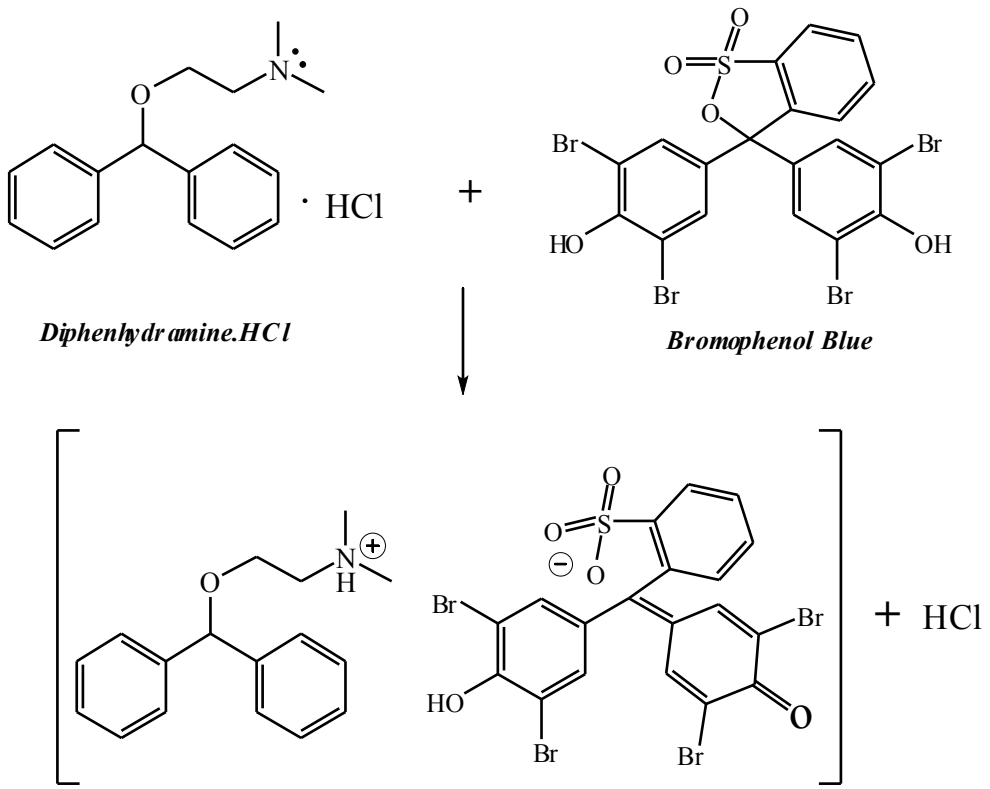
Effect of the order of addition:

The effect of order of addition of the reactants was also studied. It was found that putting reagent, buffer and finally the cited drug gave the best results instead of any other orders of addition

Stoichiometry of the complex

To establish mole ratio between diphenhydramine-HCL and BPB, Job's method of continuous variation has been used (Fig 4), the results showed that an ion-pair complex with a ratio of 1:1 was formed between the drug and BPB through an electrostatic attraction between the positive protonated diphenhydramine-HCl and the anion of BPB [22, 23] .

The formation of the ion-pair complex can be represented as in following scheme:



Ion-pair Complex

Simplex method

Simplex method used to confirm the optimum conditions which were obtained by the univariate procedure. Three major parameters (pH, reagent concentration, and shaking time) were optimized by the simplex procedure, while the other minor parameters were obtained by the univaiate method. To set simplex program for the three studied variables, four arbitrary experimental conditions should be chosen. The values of these parameters were selected within specified boundaries for each at which they affected the measured absorption signal of colored products.

The absorbencies of these four experiments were measured and the results were feed to the simplex program. Points (1 to 4) in (Table 4) represent the first four experiment cycle with their measured absorbencies. The simplex program starts to reflect the worst point through the centroid of other point to obtain a new point 5. An experiment was then performed utilizing the variable setting as a reflected point 4, the latter was rejected and replaceed by point 5. A measured absorption signal was feed again to the program and the process is repeated successively until optimum conditions were decided by the program and were similar to those obtained by the univariate method.

Calibration graph

Employing the experimental conditions, linear calibration graph for diphenhydramine is obtained (fig 5), which shows that Beer's law was obeyed in the concentration range of 0.2-25.0 $\mu g.ml^{-1}$

Spectral characteristics of the proposed method

According to the optimum experimental conditions of the proposed method, the regression plot shows a linear dependence of absorbance signals on the concentrations of the studied drug in the range given in Table 4. The regression equation, correlation coefficient, molar

absorptivity, detection limit and Sandell's sensitivity in addition to other parameter are given in (Table 5).

Accuracy and precision

The accuracy of the proposed method was confirmed by analyzing three replicate analyses of three different amounts of drug (with Beer's law) by calculating the relative error percentage (Table 6). The results indicated good accuracy of the method for Diphenhydramine-HCl. The precision was determined in each case by calculating the percentage relative standard deviation (RSD %) for three determinations at each of the studied concentration level and were found to be in the range 1.067 – 1.652 %.

Interference studies

The effect of common excipients that often accompany the studied drug in various pharmaceutical tablets were tested for possible interference in the assay. The results showed that no interference were found in the presence of 500 µg of the studied excipients (starch, Lactose, sucrose, magnesium stearat and sodium alginate) in the determination of diphenhydramine-HCl.

Analysis of dosage forms

The obtained satisfactory validation results made the proposed procedures suitable for the routine quality control analysis of diphenhydramine in commercial tablets. The results, presented in (Table 7), reveal that the values obtained for RSD% and R. E.% , indicate high accuracy and precision for the proposed method.

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Table (1): Effect of shaking time on extraction of 5µg.ml^{-1} Diphenhydramine, 0.05% BPB, pH 3.0.

Time (min.)	Absorbance
1	0.353
2	0.252
3	0.255
4	0.268
5	0.272
6	0.281
7	0.260
8	0.242

Table (2): The effect of the extraction solvent of 5µg.ml^{-1} Diphenhydramine

Organic phase	Absorbance of Drug-BPB ion pair complex
Chloroform	0.353
1,2-Dichloroethane	0.272
Benzene	0.288
Dichloromethane	0.313
Toluene	0.097

Table (3): Boundary conditions for the studied variable.

Variable	Range
pH	2.00 - 5.50
Reagent Conc. (%)	0.01 - 0.07
Shaking time (min.)	1.00 - 8.00

Table (4): Multivariate experiments (simplex) for determination of Diphenhydramine

Exp. No.	pH	Reagent Conc.(%)	Mixing Time(min.)	Abs.
1	2.5	0.04	2	0.3057
2	2.7	0.05	3	0.3071
3	3.0	0.05	1	0.3535
4	2.5	0.07	6	0.2366
5	3.0	0.03	1	0.2557
6	2.5	0.06	4	0.3280
7	3.0	0.06	2	0..2856
8	2.5	0.05	2	0.2299
9	3.0	0.05	1	0.1935
10	2.5	0.05	2	0.2299
11	3.0	0.04	1	0.2241
12	2.5	0.05	3	0.2484
13	3.5	0.05	1	0.2290
14	2.0	0.06	6	0.1787
15	3.0	0.05	2	0.2008

Table (5): Analytical characteristics for the proposed.

Parameter	BPB proposed method
Linear range ($\mu g.ml^{-1}$)	0.2 – 25.0
Regression equation	$A = 0.0828[$ Diph. $\mu g.ml^{-1}] + 2.28x10^{-6}$
Slope ($L.\ mg^{-1}.cm^{-1}$)	0.0828
Intercept	2.28×10^{-6}
Molar absorptivity ($L.mol^{-1}.cm^{-1}$)	$2.416x10^{4}$
Correlation Coefficient	0.9991
Detection Limit ($\mu g.ml^{-1}$)	0.155
Sandell's Sensitivity ($\mu g.cm^{-2}$)	0.0128

Table (6): Evaluation of accuracy and precision of the proposed method

Concentration of drug taken ($\mu g.ml^{-1}$)	Concentration of drug found ($\mu g.ml^{-1}$)	Relative Error,%	RSD , %
5	4.92	-1.60	1.067
15	15.06	+0.40	1.652
25	25.12	+0.48	1.068

Table(7):Spectrophotometric determination of Diphenhydramine in pharmaceutical compounds using Ion-pair formation

Amount of drug (μg)		Concentration ($\mu g.ml^{-1}$)		R. E.%	RSD%
Labeled	Found	Taken	Found		
25	24.48	10	9.80	-2.00	0.661
		20	19.62	-1.90	0.721
10	10.04	10	10.05	+0.50	0.409
		20	20.09	+0.45	0.387

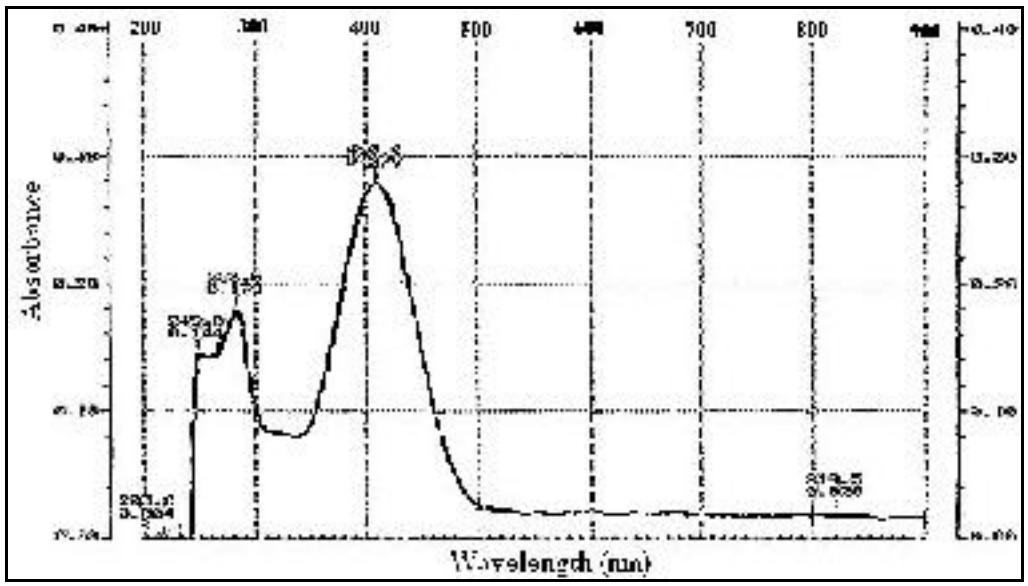

Fig.(1): Absorption spectrum of 4$\mu g.ml^{-1}$ Diphenhydramine-BPB ion-pair complex

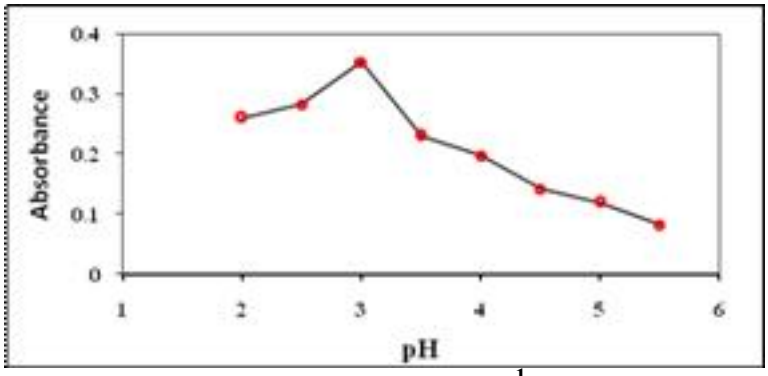

Fig.(2): Effect of pH on the absorbance of 5µg.ml^{-1} Diphenylhydramine,0.05% BPB.

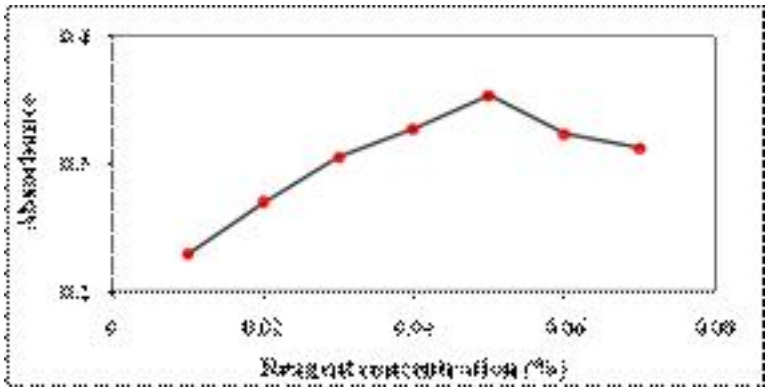

Fig.(3): Effect of reagent concentration on the absorbance of 5µg.ml^{-1} Diphenhydramine at pH 3.0.

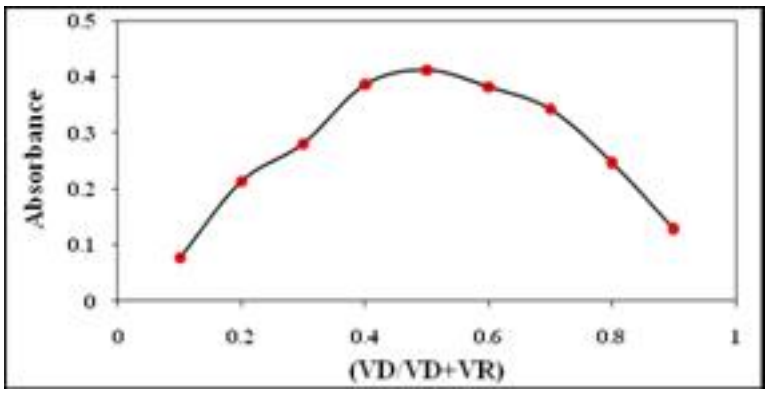

Fig.(4): Job's methods of 3.4270 x10^{-5}M (10 µg.ml^{-1}) Diphenhydramine with 1.492*10^{-3}M BPB.

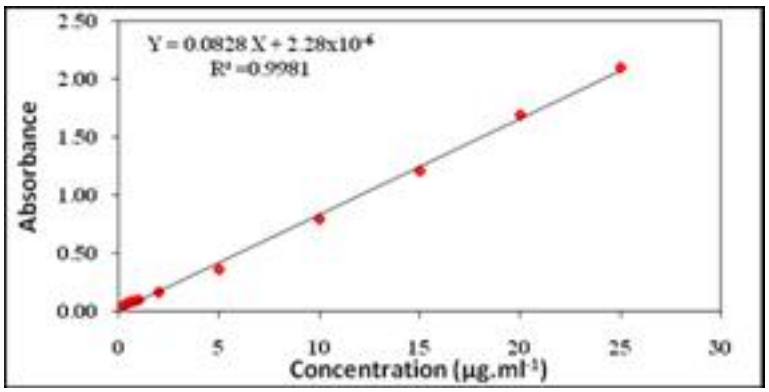

Fig.(5): Calibration graph of Diphenhydramine under optimum experimental conditions.

دراسة لايجاد الظروف المثلى بطريقتي المتغيرات الاحادية و السمبلكس

واستخدامها لتحليل ثنائي فينهيدرامين هيدروكلوريد خلال تكوين زوج ايوني

استلم البحث في: 4 آيار 2010
قبل البحث في : 3 حزيران 2010

علاء كريم محمد، سرمد بهجت ديكران، مها عبدالستار العبيدي
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الخلاصة

تم في هذا البث وصف طريقة استخلاص وتقدير طيفية سهلة وسريعة وحساسة لتحليل ثنائي فنهايدرامين – هيدروكلوريد النقي وفي بعض المستحضرات الصيدلانية. تعتمد الطريقة على تكوين معقد زوج–ايوني مع كاشف بروموفينول الازرق في وسط دارئ الفثالات (pH = 3.0) قابل للذوبان في الكلوروفورم. لقد اظهر المعقد المستخلص اعظم امتصاص عند طول موجي مقداره 410 نانومتر وكان مطاوعا لقانون بير لمدى خطي يتراوح بين (0.2- 25.0 $\mu g.ml^{-1}$) وكانت قيم معامل الامتصاص المولي وحساسية ساندل لهذا النظام هي($2.416x10^4$ $L.mol^{-1}.cm^{-1}$) و (0.012 $\mu g.cm^{-2}$) على التوالي، بينما كان حد الكشف مساويا الى (0.155 $\mu g.ml^{-1}$). لقد امكن تطبيق هذه الطريقة بنجاح لتقدير الدواء في بعض المستحضرات الصيدلانية، اذ تم الحصول على نتائج مرضية.

الكلمات المفتاحية :المتغيرات الأحادية ،السمبلكس، ثنائي فينهيدرامين هيدروكلوريد