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Spectrophotometric Determination of Paracetamol and Phenobarbital in Raw Forms and in their Pharmaceutical Preparations

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ABSTRACT
A "sensitive, rapid and economical" spectrophotometric procedures for estimation of two drugs; Paracetamol (PAR) and Phenobarbital (PHE), by reaction with 4-chloro-7-nitrobenzofurazan (NBD-Cl) as reagent in an alkaline interemediate. These methods are summarized on the forming of "color products" among these drugs and the chromogenic reagent (NBD-Cl). Yellow colored product formed at (pH 11) and λmax. 466 nm for (PAR), and yellow colored product at (pH 11.5) and λmax. 472 nm for (PHE). Beer's Law is obeyed in a concentrations range of (6-90 µg/ml), (12-80 µg/ml), with molar absorptivity (2.101×103 L/mol.cm), (3.507×103 L/mol.cm), and correlation coefficient 0.9992, 0.9988, respectively, the detection limits were (0.671 µg/ml, 2.273 µg/ml), respectively. Suggested procedure were prosperity implement to the estimation of "these drugs" in pure forms and in their pharmaceutical formulations as (Tablets).
Keywords: spectrophotometric, Paracetamol, Phenobarbital, NBD-Cl.
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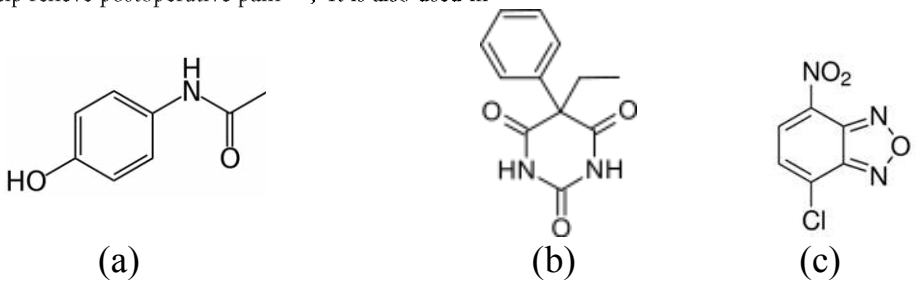
1- INTRODUCTION

1-1 Paracetamol, (Fig. 1 (a)) or Acetaminophen: is the most commonly used medication worldwide for analgesia and hypothermia, and is available over-the-counter, both in single and multicomponent preparations. It is also preferred for patients who cannot be treated with anti-inflammatory and non-steroidal drugs (NSAID), such as people with bronchial asthma, peptic ulcer disease, hemophilia, people with allergies to salicylates, children under 12, pregnant or nursing women. Chemically known is N-acetyl-para-aminophenol [1]. There are many ways in which Paracetamol is estimated, like HPLC [2,3], TLC [4,5], HPTLC [6,7], Voltammetry [8-10], Titrimetry [11], FIA [12,13], Chemiluminescence [14,15], UV-Vis. Spectrophotometry [16-18].

1-2 Phenobarbital, (Fig. 1 (b)) It is used to treat insomnia, and to help relieve postoperative pain [19], It is also used in the treatment of all types of disorders, except in cases of coma [20,21], It is not considered to be less effective than phenytones, but the tolerance and survival of phenobarbital will be less [22]. Chemically known is 5-ethyl-5-phenyl-1,3-diazinane-2,4,6-trione [23].

There are many ways in which Paracetamol is estimated, like HPLC [24,25], TLC [26], Voltammetry [27].

1-3 (NBD-Cl), (Fig.1(c))"4-Chloro-7-nitrobenzofurazan is a highly sensitive chromogenic and fluorogenic reagent" [28].



(a) (b) (c)

Fig. (1): Chemical Structures of (a) Paracetamol (b) Phenobarbital (c) NBD-Cl reagent

2- AIM OF THE STUDY

The aim of the research is to find easy, fast and inexpensive methods for estimate of Paracetamol and Phenobarbital, by "chromogenic reagent NBD-Cl" in alkaline intermediate, and these methods have succeeded in estimating Paracetamol in pharmaceutical forms as tablets.

3- EXPERIMENTAL SETUP

3-2 Materials

Paracetamol %99, Phenobarbital %99 from (SDI Samarra-Iraq), "4-Chloro-7-nitrobenzofurazan (NBD-Cl)" %98 (Solarbio), Sodium Hydroxide (NaOH) %98 (GCC), Ethanol %99.9 (Scharlau).

3-3 Preparation of Solutions

3-3-1 Paracetamol (1000 µg/ml): Prepared by weight (0.1000 gm) of (PAR), It is melted in (100 ml) ethanol.

3-3-2 Phenobarbetal (1000 µg/ml): Prepared by weight (0.1000 gm) of (PHE), It is melted in (100 ml) ethanol.

3-3-3 NBD-Cl (1×10^{-2}M): Prepared by weight (0.1996 gm) of NBD-Cl and dissolve in (100 ml) ethanol.

3.3.4 NaOH (1M): Prepared by weight (4 gm) of NaOH and dissolve in (100 ml) distilled water.

3.3.5 (Potassium sorbate, Stearic acid, Soluble starch, Aerosil, Lactose. H_2O, Magnesium stearate, Sodium lauryl sulphate and Stearic acid) solutions: a concentration of (1000 µg/mL) Prepared by weight (0.1000) gm and dissolves in 100 ml of the appropriate solvent.

4- PROCEDURES

4-1 Paracetamol: A 2.5 ml from 500 µg/mL of (PAR) was carried into 25ml "volumetric flask", 3.0 ml from 10^{-2} M "NBD-Cl" It was added and then added to it 1.0 ml from NaOH 1M. After passing (10 min.), the volume was supplemented to volume by distilled water, and then it was measured at 466 nm vs "reagent blank".

4-2 Phenobarbital: A 3.0 ml from 500 µg/mL of (PHE) was carried into 25ml "volumetric flask", 4.0 ml from 10^{-2} M "NBD-Cl" It was added and then added to it 1.0 ml from NaOH 1M. After passing (10 min.), the volume was supplemented to volume with distilled water, and then it was measured at 472 nm vs "reagent blank".

3-1 Instruments

UV-VIS Spectrophotometer/single beam/ "Genesys UV 10"; pH meter/Ino Lab pH/INO735/"Jenway 3310"; Balance Kern 770GS/GJ/"Sartorius BL210S" ; Oven/"Memmert" ; Schutzart DIN 40050-IP20.

4-3 Study steps "stoichiometric ratio"

Parity or correlation between these drugs and the reagent was studied using "molar ratio" and "continuous variation methods". In these steps, "equimolar" solutions of (PAR), (PHE) and "NBD-Cl" (5×10^{-3}M), were used. Varying aliquots of "NBD-Cl" were added to constant volumes of drugs solutions (2.5 ml from PAR and 2.0 ml from PHE), the final volumes were (25ml) absorption value was measured at 466 and 472 nm for two color products opposite the "reagent blank treated similarly". While in the latter method, a series of PAR-NBD-Cl and PHE-NBD-Cl solutions were kept at (5ml) (0 : 5, 0.5 : 4.5, 1 : 4, 1.5 : 3.5, 2 : 3, …… 5 : 0).

4-4 Applications for "proposed methods"

"Ten tablets" for each of drugs (PAR, PHE) were weighed, the averages of these weights were calculated and then crushed into a very fine powder. The weight was accurately taken amount of these powders, they were transferred to volumetric flasks of 50 ml capacity and dissolved with ethanol, and then filtered and washed, and the final volume was completed to 100 ml. "volumetric flask". To get a final concentration 1000 µg/ml. These methods were applied and succeeded in estimating of (PAR), and (PHE) In the medicines available on the market in the form of tablets.

5- RESULTS AND DISCUSSION

Absorption value of spectrum of "PAR-NBD-Cl", and "PHE-NBD-Cl" systems vs the blank in an alkaline intermedieate at room temperature (25°C) producing an "yellow colored products" for each drugs where absorbs maximally at 466 nm for (PAR), 472 nm for (PHE), (Fig. 2, 3) and reagent blank against ethanol (Fig. 4).

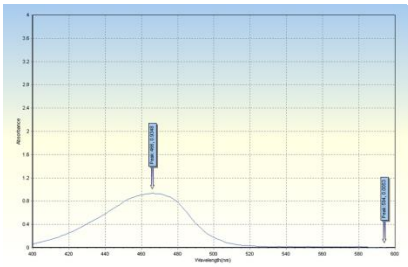


Fig. (2): Absorption spectrum of PAR-NBD-Cl system against blank

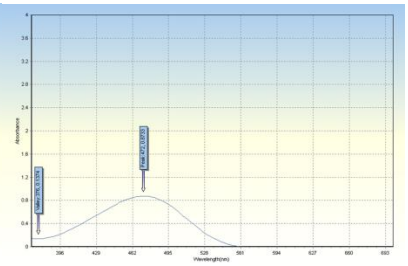


Fig. (3): Absorption value spectrum of PHE-NBD-Cl system against blank

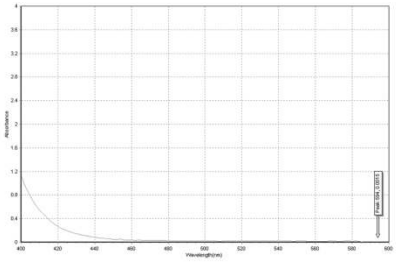


Fig. (4): Absorption value of spectrum of blank vs ethanol

5-1 OPTIMUM CONDITIONS

For establish optimum conditions, required to creation of "colored product with maximum stability and sensitivity", the influence of volumes of "NBD-Cl", and added of "alkaline intermediate", "reaction time" and the "stability of colored products" in the "room temperature (250C).

5-1-1 Study of effect of reagent volumes

Effect of reagent volumes on the reactions were studied at "room temperature". Reactions of (PAR), (PHE) with reagent were to rely on the concentrations of "NBD-Cl". So, it's concentrations were studied by different volumes from (0.5 to 7.0 ml) of (0.01 M) NBD-Cl, while the (PAR), and (PHE) concentrations were maintained constant at 50 µg/ml for (PAR) and 60 µg/ml for (PHE) (As a final concentration from 500 µg/mL as a primary concentration) for each. The color intensity was found to increase with addition of NBD-Cl up to a particular concentration and then either decrease or remain steady, the highest value of absorption intensity were attained when the volumes of NBD-Cl were (3.0 and 4.0) ml, of 0.01 M, Therefore, these concentrations were used to prepare calibration curves.

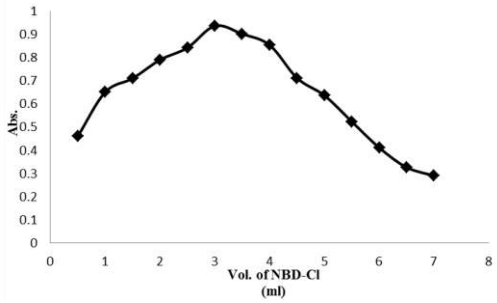


Fig. (5): Effect of conc. of NBD-Cl (0.01M) on PAR-NBD-Cl product

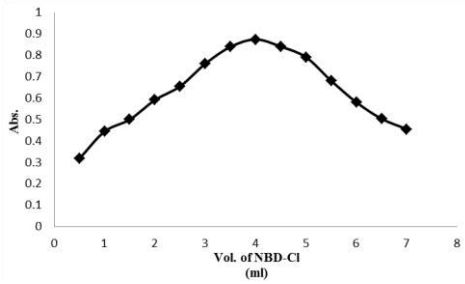


Fig. (6): Effect of conc. of NBD-Cl (0.01M) on PHE- NBD-Cl product

5-1-2 Effect of pH

An alkaline medium was required, because these drugs does not reacts with "NBD-Cl" in acidic medium, the results appeared that the absorbances value at pH < 8 were close to 0, in the acidity intermediate, these drugs have difficulty to reacts with "NBD-Cl". Different volumes and concentrations from base (NaOH) were studied, best results were at higher concentrations of NaOH (1M), with pH 11, 11.5 for (PAR), and (PHE) color products, respectively, As illustrated in Fig. (7, 8).

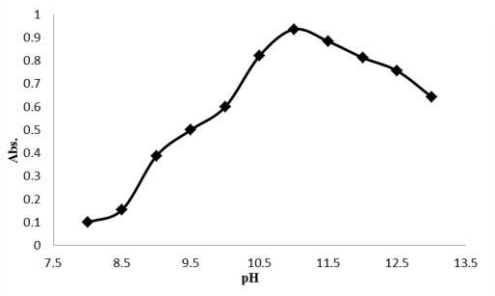


Fig. (7): Effect of pH on PAR-NBD-Cl product

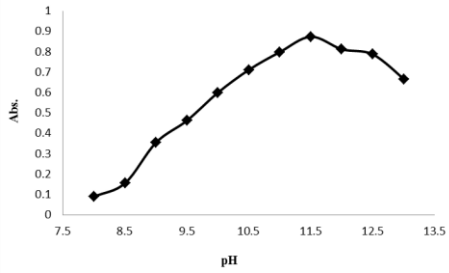


Fig. (8): Effect of pH on PHE-NBD-Cl product

5-1-3 Effect of Time

Under the "optimum conditions", the effect of reaction time of (PAR), and (PHE) with reagent in "alkaline medium" were studied, and the products were stay remained stable to 60 min. for (PAR), and to 90 min. for (PHE), As illustrated in Table (1).

Table (1): Effect of Time on color products constant

Time Min.	Abs. of (PAR) color product	Abs. of (PHE) color product
5	0.9338	0.8729
10	0.9338	0.8730
20	0.9340	0.8733
30	0.9341	0.8734
40	0.9340	0.8732
50	0.9342	0.8733
60	0.9341	0.8733
70	0.9305	0.8733
80	0.9318	0.8731
90	0.9230	0.8730
100	0.9191	0.8625
120	0.9010	0.8580

5-1-4 Study of effect of Additives

Effect of additives on the formation of the products between (PAR), (PHE) with NBD-Cl reagent the effect of adding it was studied, and not find any effect of it, as found in a table (2, 3).

Table (2): Effect of additives on (PAR) product

Additives	Added con. µg/ml	% RE	Added con. µg/ml	% RE
Potassium sorbate	250	-2.223	500	3.775
Stearic acid	250	- 1.867	500	2.541
Soluble starch	250	- 1.543	500	1.651
Aerosil	250	0.874	500	2.554

Table (3): Effect of additives on (PHE) product

Additives	Added con. µg/ml	% RE	Added con. µg/ml	% RE
Lactose.H_2O	250	- 1.684	500	- 3.681
Magnisium stearate	250	1.358	500	-1.658
Sodium lauryl sulphate	250	- 0.924	500	0.996
Stearic acid	250	-2.004	500	1.865

5-1-5 Equivalent of the reactions

In the "optimum conditions", (temperature, cons. of NBD-Cl, pH, time of stability) "the stoichiometry" of the reactions between (PAR), and (PHE) with reagent were studied by molar- ratio and continuous variation method, and the correlation ratio was between reagent and these drugs were 1:1 for (PAR), and 2:1 for (PHE) (Figs. 9, 10, 11, 12).

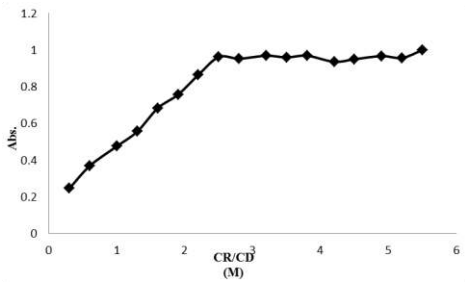


Fig.(9):Mole-ratio method of PAR

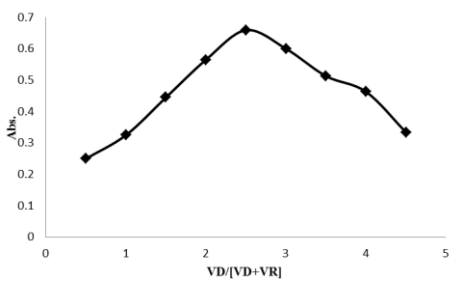


Fig.(10):Continuous variation method of PAR

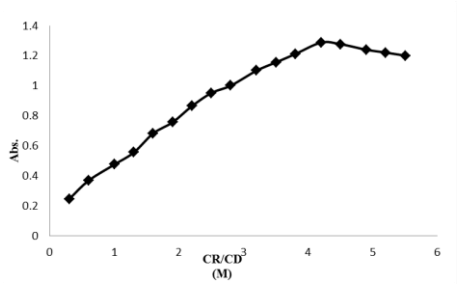


Fig.(11):Mole-ratio method of PHE

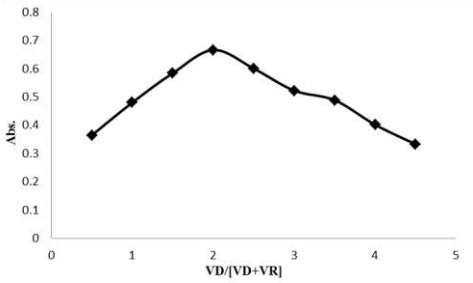


Fig.(12):Continuous variation method of PHE

5-1-6 Calibration curves

The calibration curves for (PAR), and (PHE) standard forms through correlation with NBD-Cl showed the linearity at concentrations ranges of (6-90 µg/ml), and (12-80 µg/ml), respectively, as shown in Figs. (13, 14).

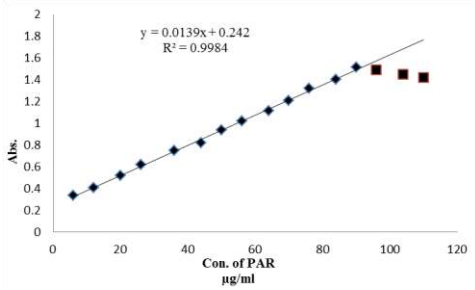


Fig. (13): Calibration curve of PAR product

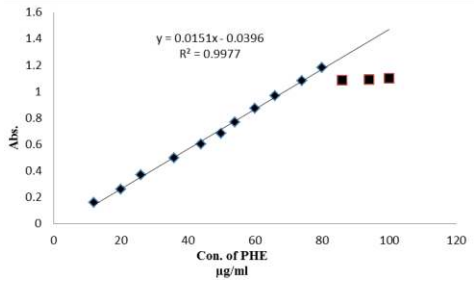


Fig. (14): Calibration curve of PHE product

5-1-7 The construction of calibration curves

The constructed according to the optimum conditions in Table (4).

Table (4): **The characteristics of the calibration curves for Determination of (PAR), and (PHE) by NBD-Cl reagent**

Parameter	(PAR)	(PHE)
$\lambda_{max.}$(nm)	466	472
Beer's law (µg/ml)	6-90	12-80
Molar absorptivity (L/mol.cm)	2.101×10^3	3.507×10^3
The correlation coefficient (r)	0.9992	0.9988
The limit of Detection (µg/ml)	0.671	2.273
The slope	0.0139	0.0151

The intercept	0.242	0.0396
% RSD	0.852	0.784

5-1-8 Study of the application for proposed methods

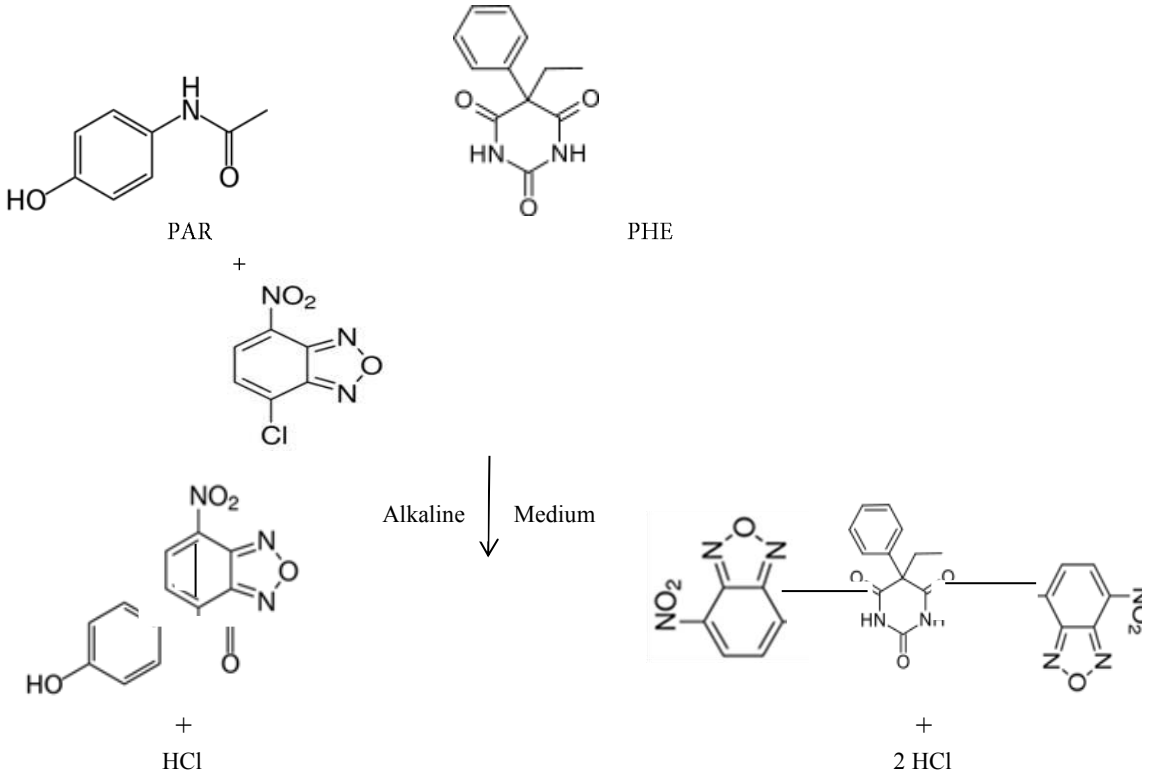
Table (5), shown the results for estimation of (PAR) and (PHE) in the pharmaceutical formulations (as tablets).

Table (5): Estimation of (PAR), and (PHE) in marketing tablets

Drug	Formulation	Content(mg)	Found(mg)	% R_E	% Rec.
(PAR)	Paracetamol	500	500.99	0.198	100.198
	Cetamol	500	499.98	-0.004	99.996
	Panadol	500	499.89	-0.022	99.978
	Dolomol	500	501.02	0.204	100.204
	Adol	500	500.76	0.152	100.152
(PHE)	Luminal	15	15.06	0.400	100.40
	Phenobarbital	15	15.18	1.200	101.20
	phenobarb	30	29.99	-0.033	99.97
	Phenobarbitone	60	60.35	0.583	100.58

5-1-9 Suggested reactions

Suggested reactions can be as in the following equations: (the drugs are associated with the reagent through the amine groups) [29,30]:



6- CONCLUSIONS

These methods described in this study is "simple, rapid, convenient" not requires You do not need special working conditions, unlike what we see in other known methods. The procedures needed shorter reactions time, Its colors are stable with a low cost reagent. The estimation can be do it at room temperature and do not require heating step. The proposed methods can be applied to determination of (PAR), and (PHE) in pharmaceutical formulations (Tablets).

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