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 inter mediate. 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], Voltammetry [7,8], Titrimetry [10], FIA [11], Chemiluminescence [12,13], UV-Vis. Spectrophotometry [14].

1-2 Phenobarbital, (Fig. 1 (b)) It is used to treat insomnia, and to help relieve postoperative pain [14]. It is also used in the treatment of all types of disorders, except in cases of coma [15]. It is not considered to be less effective than phenytoines, but the tolerance and survival of phenobarbital will be less [16]. Chemically known is 5-ethyl-5-phenyl-1,3-diazinan-2,4,6-trione [17].

There are many ways in which Paracetamol is estimated, like HPLC [4,5], TLC [10], Voltammetry [17].

1-3 (NBD-Cl), (Fig.1(c)) “4-Chloro-7-nitrobenzofurazan is a highly sensitive chromogenic and fluorogenic reagent” [17].

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

3-3-3 NBD-Cl (1 x 10³M): Prepared by weight (0.1996 gm) of NBD-Cl and dissolve in (100 ml) ethanol.

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

3.5 (Potassium sorbate, Stearic acid, Soluble starch, Aerosil, Lactose H₂O, Magnesium stearate, Sodium laurel sulphate and Stearic acid) solutions: a concentration of (1000 µg/mL) prepared by weight (0.1000 gm) and dissolve 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 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 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


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) and "NBD-Cl" (5 x 10⁻³M), 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 intermediate 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).
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.

5-1-2 Effect of pH

An alkaline medium was required, because these drugs does not react 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).
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

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

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>
<thead>
<tr>
<th>Additives</th>
<th>Added con. μg/ml</th>
<th>% RE</th>
<th>Added con. μg/ml</th>
<th>% RE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Potassium sorbate</td>
<td>250</td>
<td>-2.223</td>
<td>500</td>
<td>3.775</td>
</tr>
<tr>
<td>Stearic acid</td>
<td>250</td>
<td>-1.867</td>
<td>500</td>
<td>2.541</td>
</tr>
<tr>
<td>Soluble starch</td>
<td>250</td>
<td>-1.543</td>
<td>500</td>
<td>1.651</td>
</tr>
<tr>
<td>Aerosil</td>
<td>250</td>
<td>0.874</td>
<td>500</td>
<td>2.554</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Additives</th>
<th>Added con. μg/ml</th>
<th>% RE</th>
<th>Added con. μg/ml</th>
<th>% RE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lactose:H2O</td>
<td>250</td>
<td>-1.684</td>
<td>500</td>
<td>3.681</td>
</tr>
<tr>
<td>Magnesium stearate</td>
<td>250</td>
<td>1.358</td>
<td>500</td>
<td>1.658</td>
</tr>
<tr>
<td>Sodium lauryl sulphate</td>
<td>250</td>
<td>-0.924</td>
<td>500</td>
<td>0.996</td>
</tr>
<tr>
<td>Stearic acid</td>
<td>250</td>
<td>-2.004</td>
<td>500</td>
<td>1.865</td>
</tr>
</tbody>
</table>

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).
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).

5-1-7 The construction of calibration curves

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

<table>
<thead>
<tr>
<th>Parameter</th>
<th>(PAR)</th>
<th>(PHE)</th>
</tr>
</thead>
<tbody>
<tr>
<td>λmax (nm)</td>
<td>466</td>
<td>472</td>
</tr>
<tr>
<td>Beer's law (μg/ml)</td>
<td>6-90</td>
<td>12-80</td>
</tr>
<tr>
<td>Molar absorptivity (1/mol.cm)</td>
<td>2.101×10³</td>
<td>3.507×10³</td>
</tr>
<tr>
<td>The correlation coefficient (r)</td>
<td>0.9992</td>
<td>0.9988</td>
</tr>
<tr>
<td>The limit of Detection (μg/ml)</td>
<td>0.671</td>
<td>2.273</td>
</tr>
<tr>
<td>The slope</td>
<td>0.0139</td>
<td>0.0151</td>
</tr>
</tbody>
</table>
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).

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

5-1-9 Suggested reactions

Suggested reactions can be as in the following equations:
(the drugs are associated with the reagent through the amine group).

\[
\text{PAR} + \text{Alkaline Medium} \rightarrow \text{PAR-Cl}
\]

\[
\text{HCl} + \text{PHE} \rightarrow \text{PHE-HCl}
\]
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 done

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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