Journal of Education and Science (ISSN 1812-125X), Vol: 29, No: 3, 2020 (246-256)

Indirect Spectrophotometric Determination of Paracetamol Via Decolorization of Eriochrome Black-T With N-Bromosuccinimide

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(Received March 27, 2020; Accepted May 11, 2020; Available online September 01, 2020)

DOI: 10.33899/edusj.2020.126846.1060,

Abstract:

A simple, precise and sensitive indirect spectrophotometric method is described for the assay of Paracetamol in its pure form and pharmaceutical formulations in the aqueous medium. The method is based on the oxidation of the Paracetamol with an excess of N-Bromosuccinimide (NBS) in alkaline medium and the residual oxidizing agent bleaches the purple-colored Eriochrome black-T (EBT) to colorless species which is measured at 516 nm at room temperature. Calibration graph is linear over 0.5-9 µg mL^{-1} and molar absorptivity is 3.7×10^4 $L.mol^{-1}.cm^{-1}$. The detection and quantification limits were 0.068 and 0.229 µgmL^{-1} respectively. The accuracy (Average recovery %) is 98.57, and Precision (RSD) is ≤ 1.5. No interference effect has been observed from the excipients that exist in drug formulations. The method has been applied successfully in the determination of the Paracetamol in its commercial formulations (injection, syrup, and tablet), and compared favorably with other spectrophotometric methods used different reagents. The reaction mechanism for the oxidation of Paracetamol and EBT was postulated.

Keywords: Spectrophotometry, EBT, Paracetamol, Oxidation, NBS.

التقدير الطيفي غير المباشر للباراسيتامول بقصر لون الأريوكروم بلاك–T بواسطة N–بروموسكسينئميد

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الخلاصة:

وصفت طريقة طيفية غير مباشرة بسيطة ومضبوطة وحساسة لتقدير دواء الباراسيتامول بصيغته النقية وفي مستحضراته الدوائية في الوسط المائي. حيث اعتمدت الطريقة على أكسدة الباراسيتامول بواسطة زيادة من N–بروموسكسينئميد في الوسط القاعدي، والفائض من العامل المؤكسد يعمل على قصر صبغة الأريوكروم بلاك–T وقياس امتصاص الصبغة عند الطول الموجي 516 نانوميتر وذلك عند درجة حرارة المختبر. لقد وجد ان المنحنى القياسي خطي في مدى التركيز 0.5–9.0 مايكروغرام/مللتر، وبلغت الامتصاصية

المولارية 3.7×10^{-4} لتر/ مول.سم. وجد ان حد الكشف والحد الكمي 0.068 و 0.229 مايكروغرام/ مللتر على التوالي، ودقة الطريقة (معدل نسبة الاسترجاعية%) 98.58%، وبتوافقية (الانحراف القياسي النسبي) ≥ 1.5. كما وجد أن الطريقة لاتعاني من تداخلات من وجود مواد السواغ المضافة الى المستحضرات الصيدلانية للدواء وذلك من خلال تطبيق الطريقة بنجاح في تقدير الباراسيتامول في مستحضراته الدوائية التجارية (أمبول وشراب وأقراص)، كما تم مقارنة الطريقة المقترحة مع طرائق طيفية أخرى باستخدام كواشف مختلفة. وقد تم اقتراح ميكانيكية التفاعل لأكسدة الباراسيتامول وأيريوكروم بلاك–T.

الكلمات المفتاحية: مطياف ضوئي، أيريوكروم بلاك–T، باراسيتامول، أكسدة، N– بروموسكسينئميد

Introduction

Paracetamol also known as Acetaminophen, and chemically is *N*-(4-hydroxyphenyl) ethanamide, was first made in 1877 [1]. Paracetamol is one of the most popular drugs in the world and most used to treat fever and perhaps more common for the treatment of pediatrics. Paracetamol is also used during pregnancy and lactation [2-4], it is a harmful compound for a person's organism because it increases blood heat, and it remains active for an extended time [5]. The published spectrophotometric methods for quantification of Paracetamol within the literature are particularly relied on hydrolysis of the drug produced p-aminophenol and applying oxidative coupling [6-9] and diazotization coupling [10-12] methods using different reagents. There are also; other methods such as oxidation [13] and charge transfer complex formation reactions [14,15], or direct determination in the UV region [16-18] were described. However; some of these methods are either not sensitive and they suffer from interferences [16,17], or are carried on organic medium [14]. Other analytical techniques are additionally used for determination of Paracetamol, like HPLC [19,20], voltammetry [21,22] and chemiluminescence [23,24]. These techniques need highly sophisticated instruments. Eriochrome Black T (EBT) is an azo dye, which is known as mordant black that is used as an indicator in complexometric titrations for a metal ion determination, e.g. in the water hardness determination process. It was used for the determination of Gatifloxacin and Cefotaxime [25], Lidocaine [26], Antipsychotic Drugs [27], Dothiepin [28], Haloperidol [29], Rupatadine [30] and other drugs. However; those procedures depend on the formation of extractive ion-association complexes by organic solvents such as chloroform and methylene chloride. The present method is based on the oxidation of drugs by NBS and subsequent reaction with a fixed amount of EBT and the decrease in its absorbance was measured at 530 nm. The method needs no extraction step; it is economic and successfully applied for analysis of Paracetamol in its pharmaceutical preparations. However, a survey of literature on Paracetamol drug revealed that spectrophotometric assay based on the use of NBS as an oxidizing reagent and EBT dye as an analytical reagent had not been yet stated. The present method describes a new simple and sensitive spectrophotometric procedure for the analysis of Paracetamol in an aqueous medium, based on the oxidation of Paracetamol by NBS in basic medium and the residual oxidant bleach the color of EBT dye and measured at 516 nm.

EXPERIMENTAL

Apparatus

Absorbance and absorption spectra were recorded by a Shimadzu -1650 UV-Vis PC double beam spectrophotometer supplied with pair of a 1-cm path length silica cells, pH measurements were carried on by a combined glass electrode type Philips PW-9421 pH-meter. All calculations had been completed within the computing process in Excel program.

Reagents

All reagents and chemicals used of scientific grade were given by Fluka and BDH companies. 100 μgmL^{-1} Paracetamol stock solution was prepared by dissolving a 0.01 g of pure form drug in 100 mL distilled water in the volumetric flask. The solution was kept in the refrigerator, 500 μgmL^{-1} EBT solution was freshly prepared by dissolving 0.05 g in 100 mL distilled water in a volumetric flask, 5×10^{-3} M N-Bromosuccinimide (NBS) solution was prepared by dissolving 0.089 g in 100 mL distilled water and 0.5 M Potassium hydroxide was prepared by dissolving 2.8 g in 100 mL distilled water in a volumetric flask.

Calibration graph procedure

Aliquots of pure drug solution containing 0.5-9.0 μgmL^{-1} of pure Paracetamol, transferred into a series of 10 mL calibrated flasks. To each flask, 0.6 mL KOH (0.5 M) was added, followed by 0.8 mL NBS (5×10^{-3} M) solution. The contents gently shaken and were left aside for 10 min at room temperature. Then, 2 mL of EBT solution (500 μgmL^{-1}) was added to each flask. The solutions were diluted to the mark with distilled water. The absorbance was measured at 516 nm after 10 min versus a reagent blank.

Analysis of pharmaceutical formulations

Tablet

10 tablets Paracetamol (each tablet containing 500 mg Paracetamol) were finely powdered; an accurate weighed powder equivalent to one tablet was dissolved in 50 mL distilled, then filtered and the solution was made to 100 mL with distilled water in a volumetric flask. The resultant solution was further diluted and followed the calibration graph procedure.

Injection

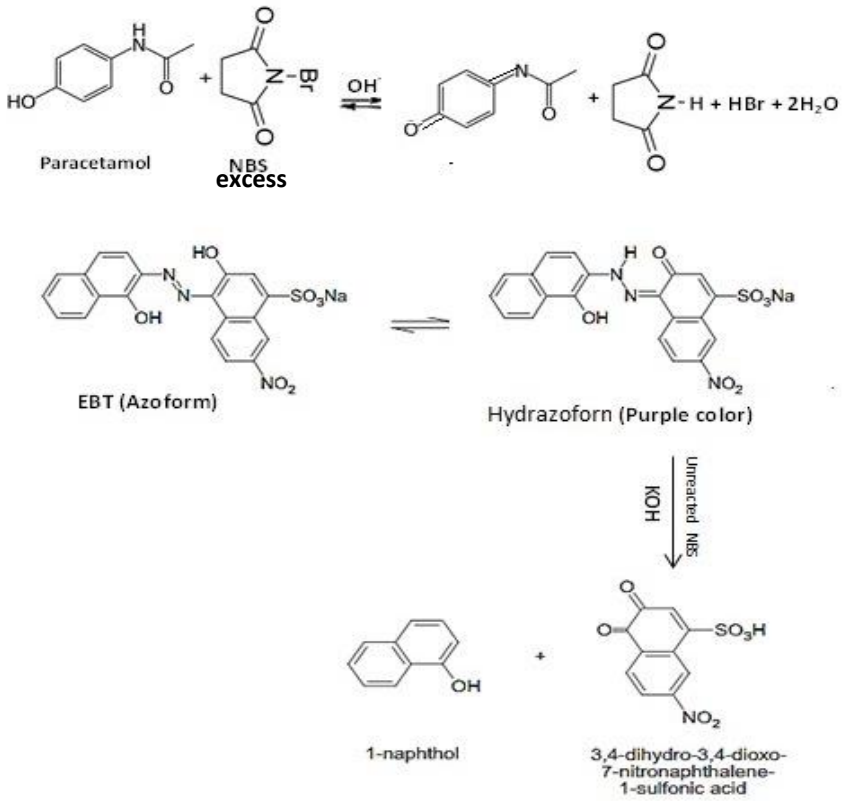
The contents of one ampoule (containing 600 mg/5mL Paracetamol) was diluted to 1L with distilled water to obtain 600 μgmL^{-1}. Further dilution was made by distilled water to get the concentration of 100 μgmL^{-1} and followed the calibration graph procedure.

Syrup

A 5 mL of antipyrol syrup (containing 0.120 g Paracetamol) was diluted with distilled water in 100 mL measuring flask to obtain 1200 μgmL^{-1}. Then further dilution was made to obtain the applicable concentration range and followed the calibration graph procedure

Results and Discussion

EBT is a darkish blue dye as powder and its solution becomes darkish purple color in distilled water. It was observed that the purple color of EBT was bleached by strong oxidizing agents. However; the proposed method primarily based on the oxidation of Paracetamol by adding a fixed amount of NBS in basic medium, and left for a specific time for the completion of the oxidation of Paracetamol. Then the unreacted NBS was reacted with known amount of EBT; the absorbance was measured at 516 nm. The decrease of NBS concentration upon oxidation of known concentration of drug lead to increase the absorbance at 516 nm; this is due to the decolorization of EBT dye by the NBS, which depends on the Paracetamol concentration. The discoloration was caused by the destruction of the dye with NBS (Scheme 1). The increasing concentration of Paracetamol, lead to decreasing in the concentration of NBS for bleaching EBT and lead to increase the absorbance at 516 nm which is proportional to Paracetamol concentration (Fig. 1).



Scheme: Proposed mechanism for oxidation of EBT by NBS

EBT showed an absorption band with λ_{max} at 516 nm in an aqueous medium (Fig. 1). The upper limit concentration of EBT was selected by plotting the absorbance of increasing amounts of dye against distilled water at λ_{max} and was found 100 µg mL^{-1} (Fig. 2) which was selected in subsequent experiments.

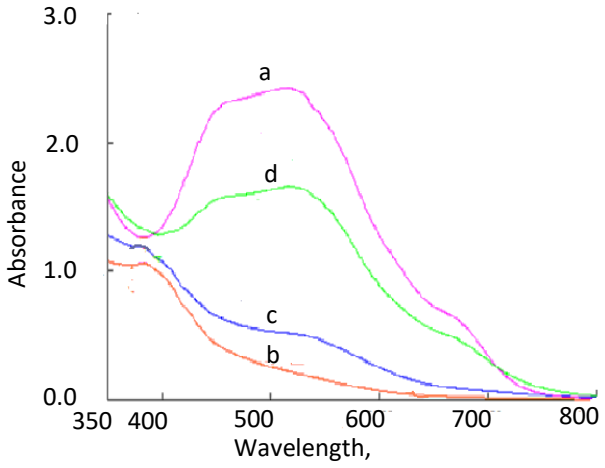


Figure1: Absorption spectra of 100 µgmL^{-1} EBT in the presence of a base (a) in the absence and (b) in the presence of 5×10^{-3}M NBS with (c)2 µgmL^{-1} and (d) 6 µgmL^{-1} of Paracetamol.

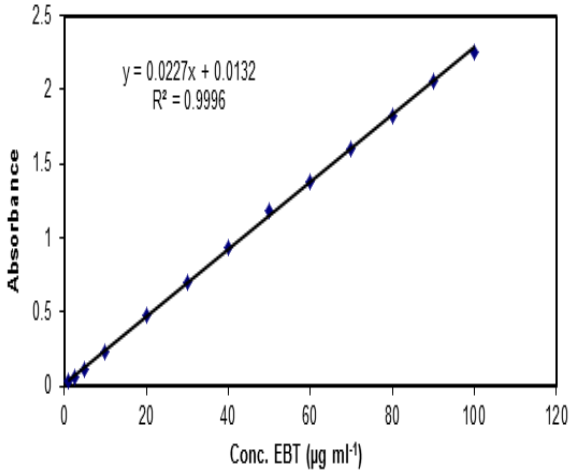


Figure 2: Calibration graph of EBT in the presence of KOH

Optimization of conditions

In order to establish the experimental conditions for high sensitivity of the method, the effect of various parameters such as oxidizing agent, base, temperature and time were studied and optimized.

Effect of oxidant and concentration

Various oxidizing agents such as potassium chromate, potassium dichromate, potassium iodate and NBS with a concentration of 5×10^{-3}M had been tested for bleaching of 100 µg mL^{-1} EBT within the presence of 1 mL of 0.5 M KOH. The reaction mixture was left for 10 min; then a volume was completed to 10 mL with distilled water and the absorbance was measured at a suitable wavelength. It was found that NBS is the best oxidant (Figure 3), and 0.8 mL of 5×10^{-3}M is sufficient to bleach the dye, (Fig. 4), which is recommended in this method.

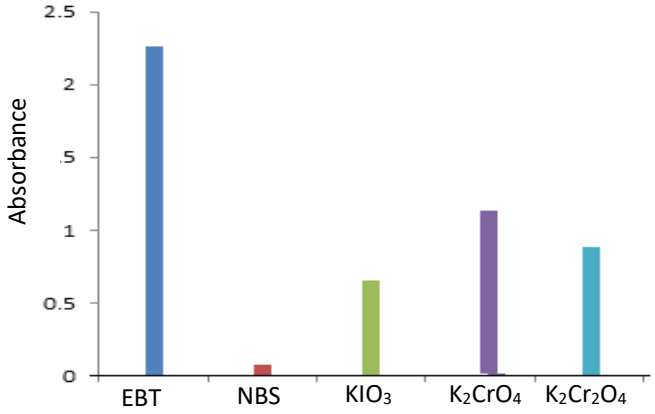


Figure 3: Effect of 1 mL of 5×10^{-3}M oxidant on the bleaching of 100 µgmL^{-1} EBT

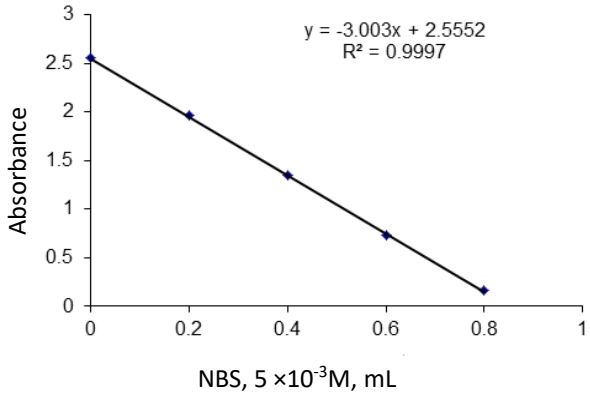


Figure 4: Effect of NBS concentration on the decolonization of 100 µgmL^{-1} EBT

Selection of base type and concentration

The oxidation of drug and dye took place in acidic and basic medium, but it was found that acid has some effect on the bleaching of EBT dye alone, whereas the base no effect. So, the oxidation processes were took place in basic medium. Various bases such as NaOH, KOH, NH4OH, and Na_2CO_3 of 0.5 M have been tested, using 2 µg mL^{-1} of Paracetamol, to obtain high sensitivity. It was also found that KOH is the best base for the system (Fig. 5). Also; it was found that 0.5 M concentration gave high absorbance (Fig. 6). The volume of 0.5 M KOH was studied and found 0.6 mL is the optimal volume (Fig. 7) and recommended in this method.

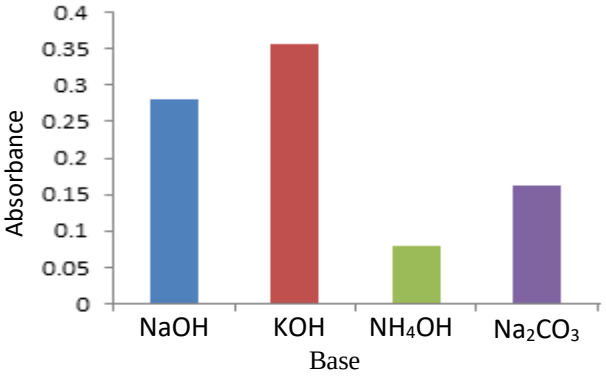


Figure 5: Effect of 1 mL of 0.5 M base on the absorbance

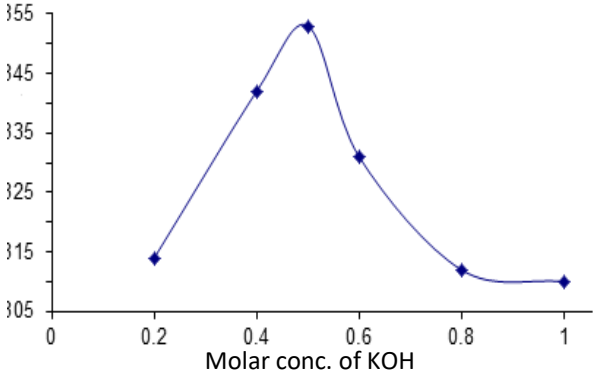


Figure 6: Effect of molar concentration of

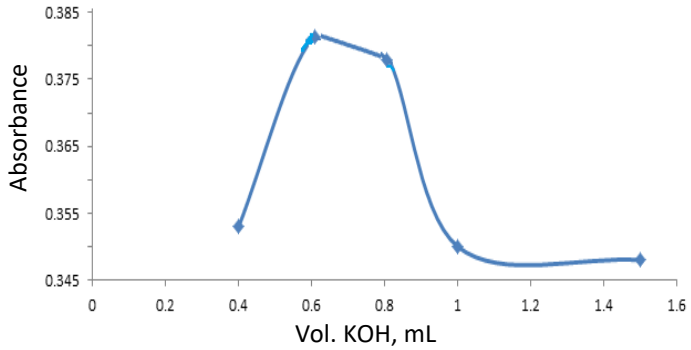


Figure 7: Effect of volume of 0.5 M KOH on the absorbance

Effect of the sequence of addition

The sequence of addition was studied; it was found that drug+KOH+NBS+EBT is the best sequence. Whereas other sequences gave lower absorbance value underneath an equivalent experimental conditions.

Effect of oxidation period

The effect of oxidation period of Paracetamol was studied by addition of 0.5 mL of 5×10^{-3}M NBS to 2 μgmL^{-1} for drug in the presence of 0.8 mL of 0.5M KOH. The solution was shaken and left at room temperature for different periods of time. After that 100 μgmL^{-1} EBT was added to the drug, and the solution was shacked and diluted to 10 mL in calibrated flask. The absorbance of the residual EBT was measured after 10 min standing time at 516 nm against distilled water. The results showed that 10 min is sufficient for the oxidation of drug and the absorbance remain constant for more than 2 hours.

Quantitation

Under the described experimental conditions, the standard calibration curve for, Paracetamol with EBT was created by plotting the absorbance versus concentration (Fig.8). Linearity limit and the molar absorptivity value were estimated. Table 1 shows that the method is sensitive, and has an excellent linearity, as the results of the regression equation and the corresponding correlation coefficient, for Paracetamol determination by the proposed method. Accuracy (average recovery %) and the relative standard deviation (RSD) for the analysis of three replicates of different concentrations for

Paracetamol explain that the method is accurate and precise. detection limit (LOD) and quantitation limit (LOQ) were calculated by the subsequent equations:

$$LOD = 3.3\ \sigma / b \qquad LOQ = 10\ \sigma / b$$

Where:

σ = standard deviation of the blank

b = slope of calibration graph.

The results, cited in Table1 are below the lower limit of Beer's law range.

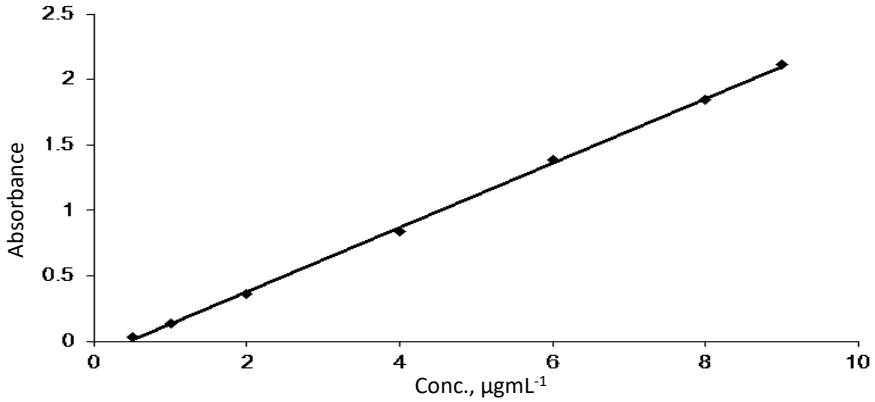


Figure 8: Calibration graph of Paracetamol

Table 1: Quantitative parameters and statistical data for assay of Paracetamol.

Parameter	Paracetamol
Linearity range (µgmL^{-1})	0.5-9
Molar absorptivity (L.mol^{-1}. cm^{-1})	3.719×10^4
Accuracy (Average recovery %)[a]	98.57
Precision (RSD)	≤ 1.5
LOD (µgmL^{-1})	0.068
LOQ (µgmL^{-1})	0.229
Correlation coefficient (R)	0.9994
Regression equation (Y)	Y = 0.246 X – 0.114
Slope	0.246
Intercept	-0.114

[a]Average of three determinations

Analysis of Paracetamol in commercial formulations

The proposed method has been applied for assay of Paracetamol in its commercial formulations (Injection, syrup, and tablet). The results cited in Table 3 indicated that the proposed method is accurate and reproducible. However; the results of the method were examined statistically by a Student's *t*-test for accuracy by applying the following equation:

$$t_{exp} = \frac{\left|\mu - \overline{X}\right| \times \sqrt{n}}{s}$$

Where µ is the certified value of the drug, $\overline{X}$, s and n are the average amount found, standard deviation for five replicates (n) respectively. The results showed in Table 2 that the experimental t-test at the 95% confidence level, were less than the theoretical value (t=2.78), indicating there is no significant

deference between certified value μ and the amount found X . Then the confidence interval for the data could be attributed to indeterminate error.

Table 2: Analysis of the Paracetamol in some commercial formulations

Drug formulation	Present amount (μgmL^{-1})	Recovery* (%)	Average recovery (%)	Drug content found	Certified Value	t-test
Injection[a]	2 8	97.15 99.59	98.37	590.22 mg/5mL	600 mg/5mL	0.261
Syrup[b]	2 8	98.17 99.28	99.02	1.18 mg/5mL	1.2 mg/5mL	1.728
Tablet[c]	2 8	96.74 99.54	98.15	490.75 mg	500 mg	2.716

* Average for three determinations

[a] London Medicals EN LTD, [b] NPI Pharma, [c] SDI-IRAQ

Selectivity

The selectivity of the proposed method has been investigated by application of the standard addition procedure for Paracetamol in commercial formulations. The obtained results in Figure 9 and cited in Table 3 indicate no effect of excipients present in formulations for assay of Paracetamol by the proposed method.

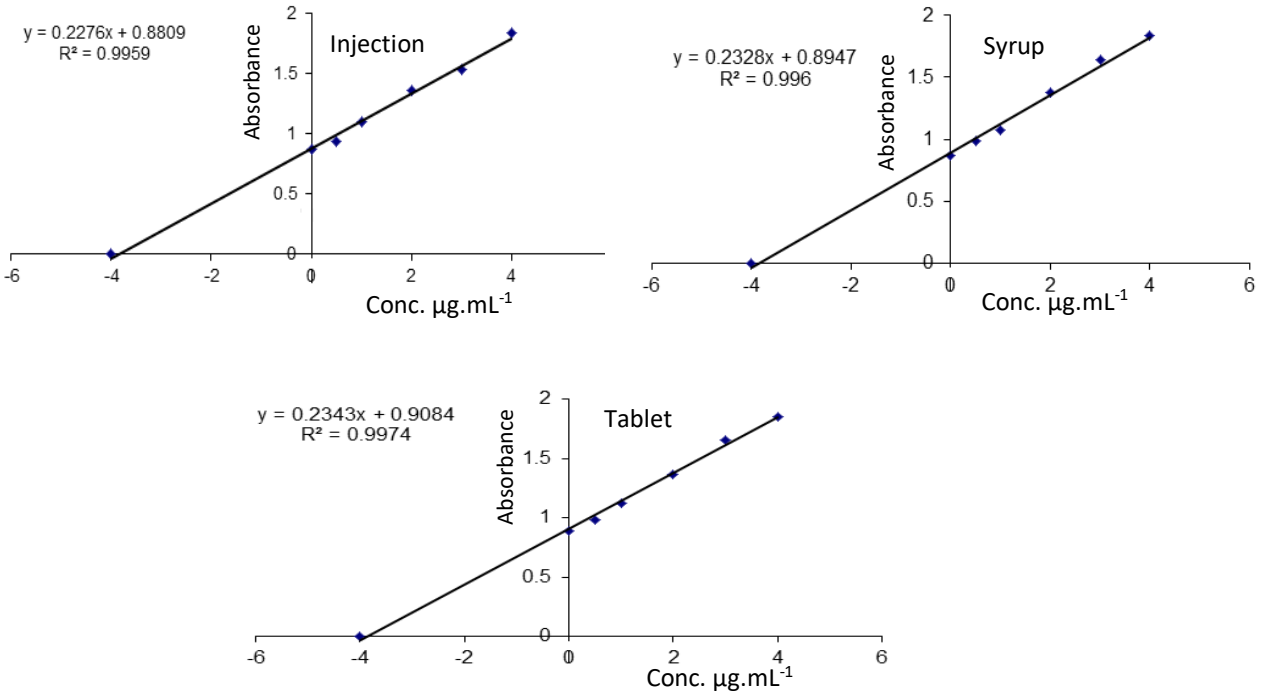


Figure 9: Standard addition procedure for determination of Paracetamol in commercial formulations

Table 3: Quantification of Paracetamol formulations by the standard addition procedure

Pharmaceutical formulation	Certified Value (mg)	Amount Taken ($\mu g.mL^{-1}$)	Recovery (%)	Drug content found(mg)
tablet	500	4	96.92	484.6
Injection	600mg/5mL	4	96.08	576.48/5mL
Syrup	1.2mg/5mL	4	96.75	1.161/5mL

Comparison of the proposed method

The proposed method has been compared with other spectrophotometric methods, described in the literature, for the determination of Paracetamol, but all of these methods cited in Table 4 suffer from limitations involving for instance, heating, hydrolysis, low sensitivity or tedious.

Table 4: Comparison of the proposed method with some literature methods

Reagent used	λ_{max} (nm)	Linearity range (μgmL^{-1})	Molar absorptivity (l/mol.cm)	Remark	Ref. no.
Water-10% methanol	243	1-30	2.00×10^3	Measurement at UV region	16
Picric acid	500	5-10	2.60×10^4	Organic medium	14
3-chloro-7-hydroxy-4-methylcoumarin	545	0-60	1.20×10^3	Involved heating to 40 ˚c for 10 min in organic medium	15
Thymol	600	1-14	6.13×10^3	Needing acid hydrolysis	31
sodium nitroprusside	700	0.19–96	3.40×10^3	Standing time 30 min at 35 ˚c	32
Proposed reagent (EBT)	516	0.5-9	3.70×10^4	Sensitive and does not involve heating or extraction	

Conclusion

A new spectrophotometric method, depending on decolorization of EBT by NBS, has been proposed for determination of Paracetamol in its pure form and pharmaceutical formulations. The method is sensitive, simple, accurate and precise, no extraction or heating is required. Moreover, the method is reliable for the determination of the drug without interference from the common excipients.

ACKNOWLEDGMENTS

The Authors would like to express their gratitude to the department of chemistry, College of Education for Pure Science, University of Mosul for using their laboratories and providing us the chemicals to carry out the research work.

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