International Journal of Advanced Chemistry Research

ISSN Print: 2664-6781
ISSN Online: 2664-679X
IJACR 2024; 6(2): 138-144
www.chemistryjournals.net
Received: 05-10-2024
Accepted: 09-11-2024

Mustafa M Abd
General Directorate of Education Anbar, Fallujah, Iraq

Abdulhakeem Dahham Hussein
Department of Applied Chemistry, College of Applied Science, University of Fallujah, Fallujah, Iraq

Omar Tareq Zaidan
Department of Applied Chemistry, College of Applied Science, University of Fallujah, Fallujah, Iraq

Corresponding Author:
Mustafa M. Abd
General Directorate of Education Anbar, Fallujah, Iraq

Spectrophotometric method for determining Paracetamol in aqueous and pharmaceutical formulations using oxidation by potassium ferricyanide and coupling with sodium tungstate

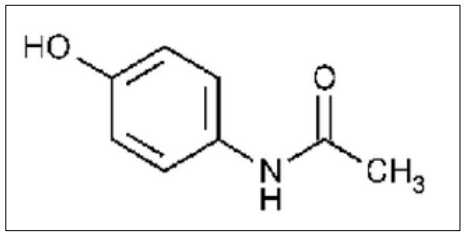
Mustafa M Abd, Abdulhakeem Dahham Hussein and Omar Tareq Zaidan

DOI: https://doi.org/10.33545/26646781.2024.v6.i2b.238

Abstract
To quantify paracetamol and its several medicinal formulations in an aqueous solution, a spectrophotometric approach was suggested. Paracetamol is oxidized by potassium ferricyanide and then coupled with sodium tungstate in a basic media as the basis for the procedure. The aqueous solution with a brown dye has the maximum absorption at 565 nm. It was discovered that the molar absorptivity was (2569.89 L/mol. cm) and that it complied with the law of beer between 1 - 65 µg/mL. The assay of pills, syrup, and ampules is accomplished with success using this technique.

Keywords: Spectrophotometric, potassium ferricyanide, paracetamol, oxidation-coupling reaction

Introduction
One of the most well-known and often-used medications for fever and pain relief is paracetamol (Acetaminophen). Among analgesics, it holds a special place. Its IUPAC name is N-(4-hydroxyphenyl) acetamide, and its formula is C8H9NO2, with a molecular weight of 151.165 g.mol-1 (Scheme 1). Many kinds of paracetamol are available, and it is frequently taken with other drugs, such as cold medications. Typically, it is taken orally or intrarectally, but it can also be infused into a vein [1].



Scheme 1: The structure of Paracetamol.

One essential component for the production of paracetamol is P-aminophenol. Additionally, it serves as an intermediate in the body's hydrolysis of paracetamol. One critical component for the production of paracetamol is P-aminophenol. Additionally, it serves as an intermediate in the body's hydrolysis of paracetamol [2, 3]. paracetamol medicinal formulations may break down into toxic substances like p-aminophenol when stored improperly [4, 5]. Numerous approaches were put proposed and documented in the literature to determine whether p-Amp was pure or the product of paracetamol's hydrolysis in an acidic or basic environment. Some of these techniques was HPLC [6, 8], electrochemical [9, 10], fluorescence [11]. Several spectrophotometric techniques have been employed because to their ease of use and the availability of several reagents and various devices that they require [12-14]. One of the most significant organic reactions with a variety of uses in analytical chemistry is oxidative coupling reactions.

The coupling of two organic molecules in the presence of an oxidizing agent under suitable conditions is necessary for oxidative coupling processes [2]. Diazotization of p-aminophenol resulted from hydrolysis of paracetamol then coupling with a various coupling agent such as 4 [(2-amino-1, 3thiazol-4-yl) amino] nitrobenzene [15].

A strong blue water-soluble dye is created when paracetamol and o-cresol undergo a direct reaction (oxidative-coupling reaction) in an alkaline media with sodium periodate present [16], Oxidative coupling techniques have also been applied to N-(1-naphthyl) ethylenediamine dihydrochloride in the presence of potassium iodate [17]. Oxidation with N-Bromo succinimide and bleaching color of Eriochrome Black-T [18], and the formation of Schiff bases also showed [19]. The present research included a spectrophotometric method for the estimation of Paracetamol in three pharmaceutical preparations in their aqueous solutions using the oxidation of Paracetamol by Potassium ferricyanide (K.F) and coupling by the detector of Sodium tungstate (STN) in an alkaline medium by Potassium hydroxide to produce brown dye.

Experimental

Apparatus

PD-303 Spectrophotometer / Apel – Japan, Shimadzu UV Spectrophotometer (UV-1800) Japanese made with 1 cm quartz cells, Sartorius / M-Power sensitive balance Japan made.

Materials

The table below shows the chemicals used in this research, along with the manufacturer and their molecular weight.

Table 1: Shows the chemicals used in this research

No	Scientific name	Molar mass (gm/mol)	Chemical formula	Processing Company
1	Paracetamol	151.17	$C_8H_9NO_2$	SDI
2	Sodium tungstate	293.82	Na_2WO_4	SDI
3	Potassium ferricyanide	329.24	$K_3Fe(CN)_6$	SDI
4	Sodium Iodate	197.89	$NaIO_3$	B.D.H
5	Potassium Permanganate	158.04	$KMnO_4$	B.D.H
6	Tris hydroxy methylamine	121.136	$C_4H_{11}NO_3$	Merck
7	Potassium dichromate	294.185	$K_2Cr_2O_7$	B.D.H
8	Potassium periodate	230	KIO_4	Merck
9	Potassium iodate	214	KIO_3	Aldrich
10	Potassium hydroxide	56	KOH	Merck
11	Sodium hydroxide	40	NaOH	Merck
12	Calcium hydroxide	79.093	$Ca(OH)_2$	Merck
13	Potassium carbonate	138.205	K_2CO_3	B.D.H
14	Sodium carbonate	106	Na_2CO_3	B.D.H
15	Potassium chromate	194.18	K_2CrO_4	B.D.H

Procedure

Standard drug solution

The standard stock solution for Paracetamol (500 ppm) was prepared by dissolving (0.05 gm) of Paracetamol in 100 ml of distilled water, then the range of concentrations (1-70 µg/mL) were prepared by subsequent dilutions of the drug.

The reagent and oxidizer solutions

The reagent of Sodium tungstate (Na_2WO_4) (STN) and oxidizer of Potassium ferricyanide (K_3Fe $(CN)_6$) (K.F) solutions were prepared by dissolving (0.2938 gm, 0.3683 gm) respectively in 100 ml of distilled water each separately to obtain a concentration of 0.01M. The solutions were saved in a dark bottle.

The base solutions

The alkaline solutions used in this work were prepared by using a concentration of (1 M) per alkaline solution by dissolving the appropriate weights in (100 ml) of distilled water.

The solutions of interfering materials: Three increasing concentrations (40, 80, 120 µg/mL) of the interfering were prepared by dissolving the following weights respectively (0.001 0.002,0.003 gm) using glucose, Lactose, Sucrose, Starch, and Sodium benzoate each separately in a 25 mL bottle, fill to the mark by distilled water.

The pharmaceutical preparation solutions

Tablets

Ten tablets of paracetamol were weighed and ground. Then precisely a weight of the mill was taken (0.0309 gm) dissolved in (100 mL) volumetric flask and fill the volume to the mark with distilled water to prepare (100 µg/mL) of paracetamol. This standard solution was used in the analytical applications of paracetamol.

Syrup and Ampule

To prepare a concentration of 100 µg/mL of paracetamol pharmaceuticals (Syrup and ampule) a (1.0416, 0.208 mL) were taken and then moved to volumetric flasks of 50 mL separately.

The overlapping materials solutions

To prepare the standard solutions of (40, 80, 120 µg/mL) of each (Starch, glucose, lactose, sucrose, sodium benzoate) by using (0.001, 0.002, 0.003 gm) of each material separately to 25mL of volumetric flasks flask and fill the volume to the mark with distilled water.

Results and discussion

For the subsequent experiments, 100 µg/mL of Paracetamol was taken using 25 mL volumetric flasks as a final volume and measured the absorbance and performed at 565 nm.

Principle of the method

This method includes the oxidation of Paracetamol by Potassium ferricyanide (K.F) and coupling by the detector of Sodium tungstate (STN) in an alkaline medium by Potassium hydroxide to produce brown dye.

Study of the optimum reaction condition: Various variables were measured to find the best color intensity for

the resulting color pigment according to the best-chosen conditions.

Study of select the best oxidizing agent

To find the best oxidizing had used for the oxidation process, (1 ml) of each of the different oxidizing agents was used separately to (1 ml) of the detector of sodium tungstate (STN) then adding (1 ml) of Paracetamol (500 µg/ml). Finally, add (1 ml) of potassium hydroxide in (25 ml) volumetric flasks complete the volume with distilled water to the mark and shake well. Potassium ferricyanide gives the best color and best intensity, so it was chosen as the best oxidizing agent for this research.

Table 2: The best oxidizing agent for the method

No	Oxidizing agent 0.01 M	Color Resulting
1	Sodium iodate	Colorless
2	Potassium Permanganate	Its color is light yellow and cloudy1
3	Tris hydroxy methyl ammine	Colorless
4	Potassium dichromate	Its color is light yellow and cloudy
5	Potassium chromate	Its color is light yellow and cloudy
6	Potassium periodate	Colorless
7	Potassium iodate	Colorless

Study of select the best volume of oxidizing agent

To find the best volume of oxidizing agent different sizes of Potassium ferricyanide were used ranging between (0.25 - 4 ml) (0.01M), then add (1 ml) of the reagent of (0.01 M) Sodium tungstate. Finally, add (1 ml) of Paracetamol (500 ppm) and (1 ml) of (1M) Potassium hydroxide. Then we complete the volume of distilled water in 25 L volumetric flasks. The 1.7 ml of Potassium ferricyanide was chosen as the best volume because it gave the highest absorption.

Table 3: The best volume of oxidizing agent

Volumes of 0.1 M Potassium ferricyanide	Absorbance
0.1 mL	0.003
0.2 mL	0.016
0.3 mL	0.034
0.4 mL	0.038
0.5 mL	0.046
0.6 mL	0.033
0.7 mL	0.041
0.8 mL	0.041
0.9 mL	0.054
1 mL	0.055
1.2 mL	0.156
1.3 mL	0.182
1.4 mL	0.202
1.5 mL	0.213
1.6 mL	0.187
1.7 mL	0.725
1.8 Ml	0.560
1.9 mL	0.521
2 mL	0.346

Study of select the best volume of the reagent

Increasing volumes of the reagent of Sodium tungstate (STN) were used at a concentration of (0.01 M), ranging between (0.1 - 2 ml). (1.7 ml) (0.01M) of oxidizing agent Potassium ferricyanide (K.F) was added, followed by the addition of (1 ml) of (500 ppm) of Paracetamol and (1ml) of (1M) Potassium hydroxide in (25 ml) volumetric flasks. The volume was completed to the mark with distilled water and well-shaken. It was found that the best volume of the detector (STN) is (0.4 ml).

Table 4: The best volume of the reagent

Volumes of 0.1 M Sodium tungstate	Absorbance
0.1 mL	0.201
0.2 mL	0.181
0.3 mL	0.168
0.4 mL	0.230
0.5 mL	0.197
0.6 mL	0.192
0.7 mL	0.183
0.8 mL	0.192
0.9 mL	0.186
1 mL	0.188
1.2 mL	0.182
1.4 mL	0.192
1.6 mL	0.187
1.8 mL	0.172
2 mL	0.174

Study of select the best base

Increasing volumes several types of alkaline solutions were used in this work, ranging from weak to strong. The base chosen is Potassium hydroxide because it gives the highest absorption and best color intensity.

Table 5: The best base

The using base 1 M	Absorbance	Color
NaOH	0.185	Orange brown
$Ca(OH)_2$	0.173	Very light yellow color
KOH	0.230	Dark brown color
K_2CO_3	0.074	Very light yellow color
Na_2CO_3	0.051	Very light yellow color

Study of select the best volume of KOH

To measure the best volume of Potassium hydroxide, absorbents of several volumes ranged between (0.1 – 1 ml). The (0.2 ml) was the best volume among the other because it gave the highest absorption and best color intensity.

Table 6: The best volume of KOH

Volumes of 0.1 M KOH	Absorbance
0.1 Ml	0.218
0.2 mL	0.242
0.3 mL	0.205
0.4 mL	0.198
0.5 mL	0.185
0.6 mL	0.203
0.7 mL	0.242
0.8 mL	0.195
0.9 mL	0.198
1 mL	0.208

Study of the effect of sequence of additions

To study the effect of the sequence of additions on the absorption and intensity of the resulting color, several sequences were taken. It was found that the best sequence is when the reagent is added first, followed by the oxidizing agent, then the drug, and finally, the base is added.

Table 7: The effect of a sequence of additions

Order	Sequence	Absorbance
1	K.F + STN +D + B	0.218
2	STN + K.F + D + B	0.242
3	D + STN +K.F + B	0.205
4	D + K.F + STN + B	0.198
5	K.F + D + STN + B	0.185
6	STN + D + K.F + B	0.203

*D= Drug, B =Base, STN = detector of Sodium tungstate, K. F= oxidant agent of Potassium ferricyanide

Study of effect of the time of oxidation

To study the effect of settling time on the absorption of the product from the oxidation process, appropriate sequences of additions were applied. The resulting dye of oxidation was stable for more than (50 minutes), while the best time for waiting for measure is (5 minutes).

Table 8: The effect of the time of oxidation

Time	Absorbance
5	0.310
10	0.308
15	0.302
20	0.286
25	0.270
30	0.258
35	0.240
40	0.235
45	0.220
50	0.201
One hour	0.188

Table 9: The summary of ideal conditions

λ_{max}	Temp (c°)	Stability period (min)	Reagent Vol	Oxidant agent Vol.	KOH 1 M Volume	Suitable solvent
565nm	25 C°	5 min	0.4 mL	1.7 mL	0.2 mL	Distilled Water

Final absorption spectra

A brown dye was formed that gave the highest absorption at 565 nm. This dye is produced by the oxidation of Paracetamol by Potassium ferricyanide (K.F) and coupling by the detector of Sodium tungstate (STN) in an alkaline medium by Potassium hydroxide. Absorbance was measured using distilled water as a good solvent for this method.

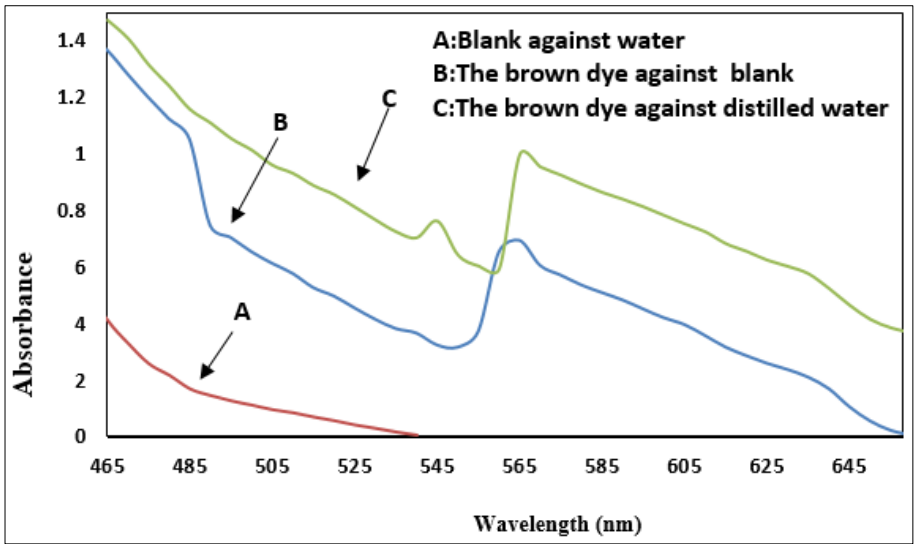


Fig 1: Absorption spectrum of Paracetamol at 483 nm by Uv-Visible

Procedure and calibration graph

To find out the calibration graph for this research, (25 ml) volumetric flasks were used (0.4 ml) of (0.01 M) of the reagent Sodium tungstate (STN) was added to all volumetric flasks and then (1.7 ml) of (0.01M) the oxidant Potassium ferricyanide (K.F) was added. Later, increasing volumes of (500µg/ml) Paracetamol were added, ranging between (0.05 – 3.75 ml). Finally, (0.2 ml) of (1 M) KOH was added to all volumetric flasks and made up to the mark with distilled water and shaken well. The resulting aqueous solutions of the dye were waited for (5 min) and then their absorbance was measured at (565 nm) against the blank. The resulting linearity limits ranged between (1 - 65 µg/ml) and the molar absorptivity was (2569.89 L/mol. cm).

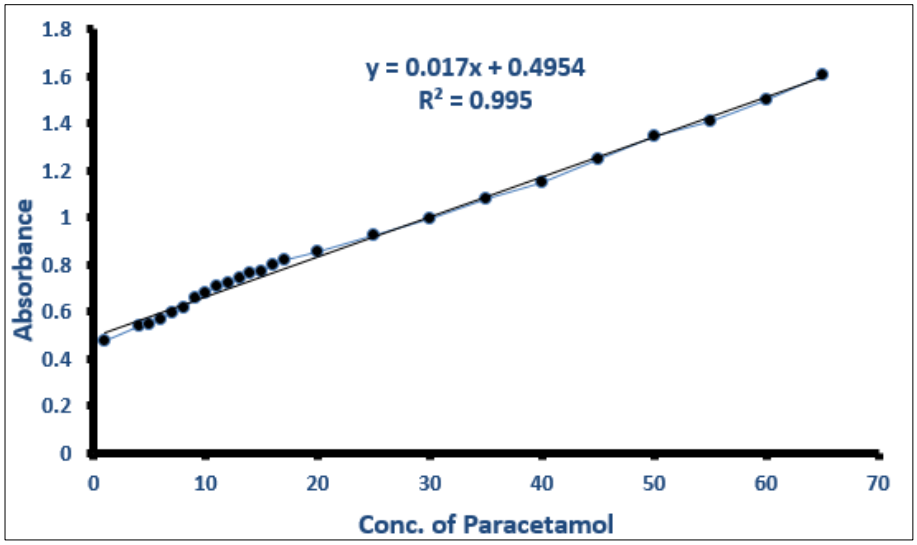


Fig 2: The calibration curve of Paracetamol at 565 nm

Table 10: Calibration graphs for the determination of Paracetamol by this method

Slope	The calibration coefficient R^2	Linearity range (µg/mL)	Molar absorptivity (L/mol.cm)	LOD (µg/mL)	LOQ (µg/mL)	Sandell Index (µg/mL)
0.017	0.9950	1- 65	2569.89	0.229	0.764	0.05882

Accuracy and Precision

To find the accuracy and precision found in this research, three different concentrations were proposed. The results for the accuracy values were very satisfactory and ranged between (96% - 102.6%) and the precision was high.

Table 11: The recovery and average percentage and precision of the method

Amount taken µg/mL	Abs	Amount found µg/Ml	Rec%	Avg of Rec%	RSD
7	0.610	6.74	96%	96%	0.0007%
7	0.610	6.74	96%		
7	0.611	6.8	97%		
25	0.934	25.8	103%	102.6%	0.0083%
25	0.930	25.5	102%		
25	0.929	25.5	102%		
40	1.162	39	98%	97%	0.00094%
40	1.157	38.97	97%		
40	1.157	38.97	97%		

Nature of dye: To find out how the drug of Paracetamol is related to the reagent used, the Job method was applied. The resulting ratio for this work to the brown dye Paracetamol was (1:1).

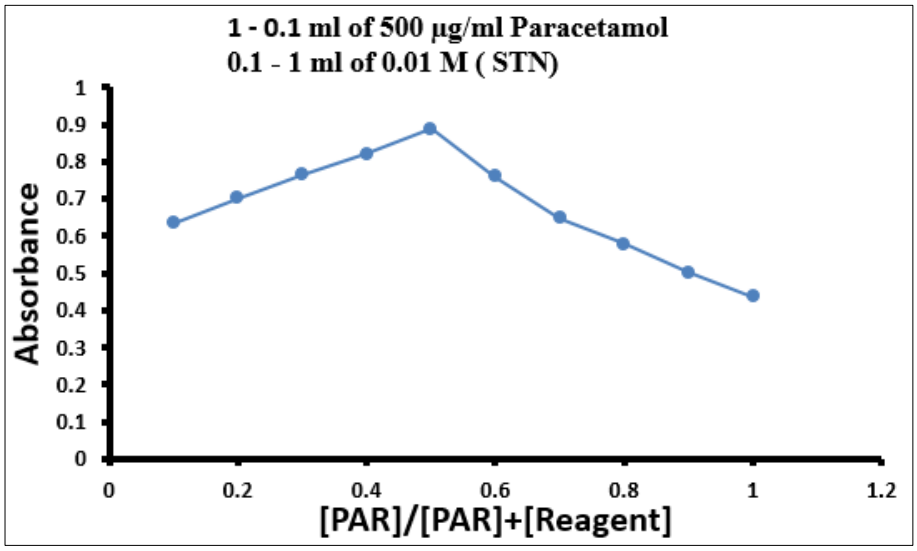


Fig 3: The application of Job`s method

The effect of interference: To study the effect of some interfering substances on the composition of the resulting brown dye, three different concentrations of them were used with a fixed concentration of Paracetamol. The solutions were used in final volumetric flasks of 25 ml and gave the same brown dye with high accuracy values.

Table 12: The summary of the effect of excipients

Interferences	Conc. µg/mL	Conc. Taken of paracetamol µg/mL	Abs	Conc. Found of paracetamol µg/mL	Rec%
Glucose	40	25	0.930	25.4	101%
	80	25	0.928	25.5	102%
	120	25	0.928	25.5	101%
Lactose	40	25	0.931	25.6	102%
	80	25	0.929	25.5	102%
	120	25	0.930	25.4	101%
Sucrose	40	25	0.925	25.2	101%
	80	25	0.930	25.4	101%
	120	25	0.927	25.3	101%
Starch	40	25	0.925	25.2	101%
	80	25	0.929	25.5	102%
	120	25	0.928	25.4	101%
Sodium benzoate	40	25	0.920	24.9	99.9%
	80	25	0.920	24.9	99.9%
	120	25	0.925	25.2	101%

Application of the method on the pharmaceutical dosage

The direct method was applied to three pharmaceutical preparations of Paracetamol in their aqueous solutions and all of them gave high accuracy values and satisfactory results. A concentration of 500 µg/ml of each pharmaceutical preparation was prepared and the method was applied in final (25 ml) volumetric flasks.

Table 13: The used pharmaceutical preparation and its concentration & origin

Drug	Pharmaceutical content	Concentration	Company
Paracetamol	Paracetamol tablets	500 mg	Aswar AL-Khaleej Company
Paracetamol	Antipyrol syrup	120 mg /5 ml	SDI -Iraq
Paracetamol	Paracetamol ampule	600mg / 5 ml	SDI -Iraq

Table 14: The use of pharmaceutical application dosage

Pharmaceutical Formula	Conc. Taken of paracetamol µg/mL	Conc. Found of paracetamol µg/mL	Rec%	Avg. Rec%
Tablets 500 mg	10	9.68	96%	96.6%
	30	29.38	97%	
	50	48.5	97%	
Ampule 500mg/ 5ml	10	9.68	96%	97%
	30	29.27	97%	
	50	49.56	99%	
Syrup 120 mg/ 5ml	10	10.3	103%	101%
	30	29.5	98%	
	50	51.4	102%	

Conclusion

The oxidation and conjugation were successfully applied to Paracetamol by spectrophotometric technique (Uv-vis). This procedure gave a good linearity which ranged between (1- 65 µg/ml) and molar absorptivity equal to (2569.89 L/mol.cm) and Sandell`s index (0.05882 µg/ml). This method has been successfully applied to three types of pharmaceutical preparations. This proposed method gave high accuracy and high precision and gave a detection limit equal to (0.229 µg/ml) and a quantitative limit equal to (0.764 µg/ml).

Comparing the Suggested Approach to Literature Methods

Other oxidative coupling reaction methods were compared with the suggested approach for estimating Paracetamol; the results are shown in the table.

Table 15: The advantages and distinctions between the suggested approach and others in the literature are emphasized by this comparison.

Analytical Parameter	Literature Method [20]	Literature Method [2]	Present method
Reagent	Copper sulphate	Methyldopa	Sodium tungstate
Beers law	(2-100) µg/ml	(10-100) µg/ml	(1-65) µg/ml
λ max	520 nm	580 nm	565 nm
Molar absorption coefficient	$8.61 * 10^{2}$ L/mol.cm	$7.75 * 10^{3}$ L/mol.cm	2569.89 L/mol.cm
Solvent	Distilled water	Distilled water	Distilled water
Recovery	99.2%	98.9%	98.5%
RSD	0.484	0.9961	0.0033
Sandell index	$6.62 * 10^{-6}$ µg/ml	----------	0.05882 µg/ml
pharmaceutical preparation	Tablets	Tablets, Syrup, Ampule	Tablets, Syrup, Ampule

References

1. British Pharmacopoeia. 2024. 5th ed. London: The Stationery Office; Copyright by System Simulation Ltd.
2. Thanoon ES, Raheed LA, Hasan IY. Oxidative coupling-base reaction for the indirect spectrophotometric determination of paracetamol in drug formulations. Int. J Eng Sci Technol. 2021;3(1):72-78.
3. Sandrina S, Fauzan A. The process of synthesizing paracetamol involves the utilization of acetic anhydride to acetylate p-aminophenol. Crown J Dent Health Res. 2023;1(2):28-31.
4. Soliman SS, Rahman MAA. Sustainable chemometric analysis of tension headache drugs in the presence of nephrotoxic impurities. Sustain Chem Pharm. 2024;42:101785.
5. Mgharbel M, Halawy L, Milane A, Zeaiter J, Saad W. Pyrolysis of pharmaceuticals as a novel means of disposal and material recovery from waste for a circular economy. J Anal Appl Pyrolysis. 2023;172:106014.
6. Hemant KT, Krishna PCK, Varaprasad RK, Srinivasa RY. RP-HPLC method for estimation of tramadol hydrochloride and paracetamol in pharmaceutical formulation. GSC Biol Pharm Sci. 2019;8(1):1-7.
7. Mohammed OJ, Hamzah MJ, Saeed AM. RP–HPLC method validation for simultaneous estimation of paracetamol and caffeine in formulating pharmaceutical form. Res J Pharm Technol. 2021;14(9):4743-4748.
8. Khosroshahi AM, Aflaki F, Saemiyan N, Abdollahpour A, Asgharian R. Simultaneous determination of paracetamol, 4-aminophenol, 4-chloroacetanilid, benzyl alcohol, benzaldehyde and EDTA by HPLC method in paracetamol injection ampoule. J Pharm Heal Sci. 2016;4(1):61-69.
9. Keskin E, Ertürk AS. Electrochemical determination of paracetamol in pharmaceutical tablet by a novel oxidative pretreated pencil graphite electrode. Ionics. 2018;24:4043-4054.
10. Masood Z, Muhammad H, Tahiri IA. Comparison of different electrochemical methodologies for electrode reactions: A case study of paracetamol. Electrochem. 2024;5(1):57-69.
11. Li L, Lu Y, Ding Y, Cheng Y, Xu W, Zhang F. Determination of paracetamol based on its quenching effect on the photoluminescence of CdTe fluorescence probes. J Fluoresc. 2012;22:591-596.
12. Behera S, Ghanty S, Ahmad F, Santra S, Banerjee S. UV-visible spectrophotometric method development and validation of assay of paracetamol tablet formulation. J Anal Bioanal Tech. 2012;3(6):151-157.
13. Hussein AD, Abdulrahman MT, Raheem AS. UV spectrophotometric development and HPLC methods for estimation of paracetamol in oral syrup formulation: Validation, comparison and application. Int J Psychosoc Rehabil. 2020;24(04):1-12.
14. Fakayode SO, Bolton B, Dassow B, Galvez K, Chohan H. Rapid screening and multicomponent quantifications of active components of oral syrup over-the-counter medications by Raman and UV–visible spectroscopy and multivariate regression analysis. Spectrochim Acta A Mol Biomol Spectrosc. 2024;305:123447.
15. Abbas R, Allawi A, Abdulhassan N, Mahmoud N. Spectrophotometric determination of paracetamol using a newly synthesized chromogenic reagent 4-[(2-amino-1, 3-thiazol-4-yl) amino] nitro benzene. Egypt J Chem. 2020;63(12):4681-4693.
16. Al-Abachi MQ, Al-Safi SA, Al-Ward HS. Spectrophotometric kinetic methods for the determination of paracetamol in pure form and pharmaceutical preparations. Iraqi J Sci. 2015;56(4A):2704-2717.
17. Othman NS. Estimation of p-aminophenol via diazotization and coupling reaction with 4-chlororesorcinol–application on paracetamol. Rafidain J Sci. 2022;31(1):35-46.
18. Al-Sabha T, Al-Gubouri Z. Indirect spectrophotometric determination of paracetamol via decolorization of Eriochrom Black-T with N-bromosuccinimide. J Educ Sci. 2020;29(3):246-256.
19. Mohamed M, Abdelakder H, Abdellah B. Microwave assisted synthesis of 4-aminophenol Schiff bases: DFT computations, QSAR/Drug-likeness properties and antibacterial screening. J Mol Struct. 2021;1241:130666.
20. Aljeboree AM, *et al*. Validated spectrophotometric approach for determination of paracetamol in pure and pharmaceutical dosage forms using oxidative coupling reaction. AIP Conf Proc. 2022;2386(1):1-7.