Rafidain Journal of Science
https://rsci.mosuljournals.com

Vol. 31, No.1, pp. 35-46, 2022

Estimation of p-Aminophenol via Diazotization and Coupling Reaction with 4-Chlororesorcinol –Application on Paracetamol

Hiba A. Alhfidh
Department of Chemistry/ College of Education for Pure Science/ University of Mosul
Nabeel S. Othman
Department of Chemistry / College of Science/ University of Mosul

Article information

p-ISSN: 1608-9391
e -ISSN: 2664-2786

Received: 23/8/2021
Accepted: 3/10/2021

DOI:
10.33899/rjs.2022.172930

corresponding author:
Hiba A. Alhfidh
hibaabed34@yahoo.com

Nabeel S. Othman
nsn20002004@uomosul.edu.iq

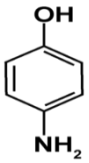
ABSTRACT

A spectrophotometric method has been proposed for the direct determination of p-aminophenol (p-Amp). The method includes diazotisation of p-Amp using an excess of sodium nitrite and in the presence of hydrochloric acid, and after destroying the remaining nitrous acid by adding sulphamic acid, then the produced diazonium salt coupled with the reagent 4-chlororenocinol (4-Chlr) in an alkaline medium of sodium hydroxide to form a water-soluble azo dye that gives the highest absorption at the wavelength of 488 nm. The linear range that follows Beer's law was from 0.5 to 15 µg/ml. The value of the molar absorptivity was 1.9905×10^4 l/mol. cm. After investigation of the optimal conditions by studying all factors affecting the absorption of the formed azo dye. The method was applied to estimate paracetamol in its pharmaceutical preparations after hydrolysis process. The results of standard addition method proved that there is no interference by additives.

Keywords: p-aminophenol, the diazotisation and coupling reaction, 4-chlororesorcenol, paracetamol.

INTRODUCTION

p-Aminophenol is an important organic compound. Its slightly hydrophilic character, moderately soluble in alcohols, and it oxidizes readily. The IUPAC Name is 4-aminophenol($H_2NC_6H_4OH$) and has the following structure (Scheme 1) (Mitchell and Waring, 2002).



M.Wt =109.128 g/mol

Scheme 1: The structure of p-Amp.

Paracetamol (acetaminophen) is the safer analgesic and is used as a single drug or combined with other drugs in various formulations (tablets, injection, syrups, and suppositories (British Pharmacopeia, 2009). Paracetamol is undergoing acid or basic hydrolysis to produce p-Amp) (Younis and Othman, 2018).

Several techniques or methods were proposed and listed in literature for estimating p-Amp as pure or the result from acidic or basic hydrolysis of paracetamol. These methods included HPLC) (Šatínský *et al*., 2013; Crevar *et al*., 2008; Khosroshahi *et al*., 2016), electrochemical (Jamal *et al*., 2004; Singh *et al*., 2019), flow injection (Bloomfield, 2002), fluorometric (Dejaegher *et al*., 2008). A large number, not a few, of spectrophotometric methods have been used according to their simplicity and availability of their requirements of different devices and a large number of reagents that can interact with the two functional groups (hydroxyl and the amine) of the compound under study. The spectrophotometric methods included various types of reactions such as 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 (Abbas *et al*., 2020), histidine reagent in alkaline medium (Alubaidy *et al*., 2019), 1-naphthol (Iorhemen *et al*., 2017), 2, 7-dihydroxy naphthalene in alkaline medium (Younis and Othman, 2018), hydroxyl analog of the pharmaceutical naproxen (Mahmood, 2020), phloroacetophenone in an alkaline medium (Othman, Zakaria, 2007). The oxidative coupling methods have been used with a different reagents such as using N-(1-naphthyl) ethylendiamine dihydrochloride in presence of potassium iodate (Othman and Zakaria, 2009), and 2, 5-di-hydroxy benzaldehyde in presence of potassium periodate as oxidizing agent (Younis and Othman, 2018), oxidation and bleaching color of dye: oxidation with N-bromosuccinimide and bleaching color of rifampicin (Younis and Othman, 2019), with N-bromosuccinimide and bleaching color of Eriochrom Black-T (Al-Sabha and Al-Gubouri, 2020) and the Schiff bases formation also presented (Mohamed *et al*., 2021).

The present research included a spectrophotometric method for estimation of p-amino phenol in aqueous solution using diazotization and coupling with 4-Chlr as a coupling reagent in alkaline medium and an application part included determination of paracetamol in different pharmaceutical formulations.

EXPERIMENTAL

Apparatus

Spectrophotometric measurements and absorption spectrum were carried out using a Jasco V-630 spectrometer. Glass and quartz cells were used with a 1 cm light bath. The acidity function of the solutions was measured using the HANNA pH 211 microprocessor pH meter and also using a BEL-type sensitive balance.

Chemicals used

All chemicals used are in a high degree of purity.

Solutions used

Para-aminophenol solution (100 µg/ml)

This solution was prepared by dissolving 0.0100 g of the pure p-Amp in 5 ml of ethanol, then transfer to a volumetric flask of 100 ml and the volume completed to the mark with distilled water.

4-Chlororesorcinol solution (0.1%)

0.1 g of 4-Chlr is dissolved with distilled water in a volumetric flask of 100 ml.

Other solutions

The aqueous solutions were prepared by weighting the accurate weight and dissolved in distilled water in volumetric flasks, these solutions included:

1% sodium nitrite, 3% sulphamic acid and 1M sodium hydroxide, and also1M hydrochloric acid was prepared by suitable dilution of concentrated acid.

Pharmaceutical Preparations Solutions

Paracetamol tablets

10 tablets (each tablet contains 500 mg of paracetamol) carefully crushed after weighing each one. A 0.327 g of the powder of paracetamol which is equivalent to 0.250g of pure paracetamol weighed and dissolved in ethanol (10 ml), then add 75ml of distilled water, filtered into a volumetric flask of 250 ml and complete with distilled water to the mark. Transferred 150 ml to round bottom flask with 25 ml of concentrated hydrochloric acid and placed for a process of reflux for one hour. Then cooling the solution, and neutralized the solution by adding 20% sodium carbonate and diluted to 250 ml with distilled water (Othman; Zakaria, 2007). A dilution from the above solution to prepare solution with a concentration of 100 µg /ml.

Paracetamol Injection Solution

A solution with a concentration of 100 µg / ml was prepared by drawing a volume of 2.09 ml (equivalent to 0.250 mg) from an injection containing 600 mg in every 5 ml, mixed with 25 ml of concentrated hydrochloric acid and placing it in a round bottom flask for a process of reflux for an hour. A similar procedure cited above for the preparation of paracetamol tablet solution has been followed and a solution of 100 µg / ml was prepared, and different volumes were taken from the final solution to obtain diluted concentrations.

Procedure and Calibration Curve

The standard curve was prepared by adding 0.6 ml of hydrochloric acid solution(1M) to various volumes of p-Amp (50 µg/ml) into a series of volumetric flasks of 10 ml to cover the concentration range from 0.5 to 15 µg/ml then 0.1 ml of sodium nitrite solution (1%), wait for 1 minute with shaking, then add 0.2 ml sulphamic acid (0.3%), waiting for two minutes with shaking, followed by adding 2 ml of 4-Chlr reagent (0.1%), and 1 ml of 1M of sodium hydroxide solution. Then, the absorbance was measured at 488 nm after dilution with distilled water to the mark. The linear range of the concentration is 0.5 to 15 µg/ml and there is a negative deviation from Beer's law after the upper limits Fig. (1). The value of the determination coefficient of the standard curve was 0.9963, which statistically indicates that it has excellent linear characteristics, and the molar absorptivity was is equal to 1.9905×10^4 l /mol.cm and Sandell's index value is 0.00548µg/cm^2, which indicates the high sensitivity of the method. Limits of detection (LOD) and limit of quantitation, LOQ were calculated and equal to 0.016 µg/ml and 0.054 µg/ml respectively.

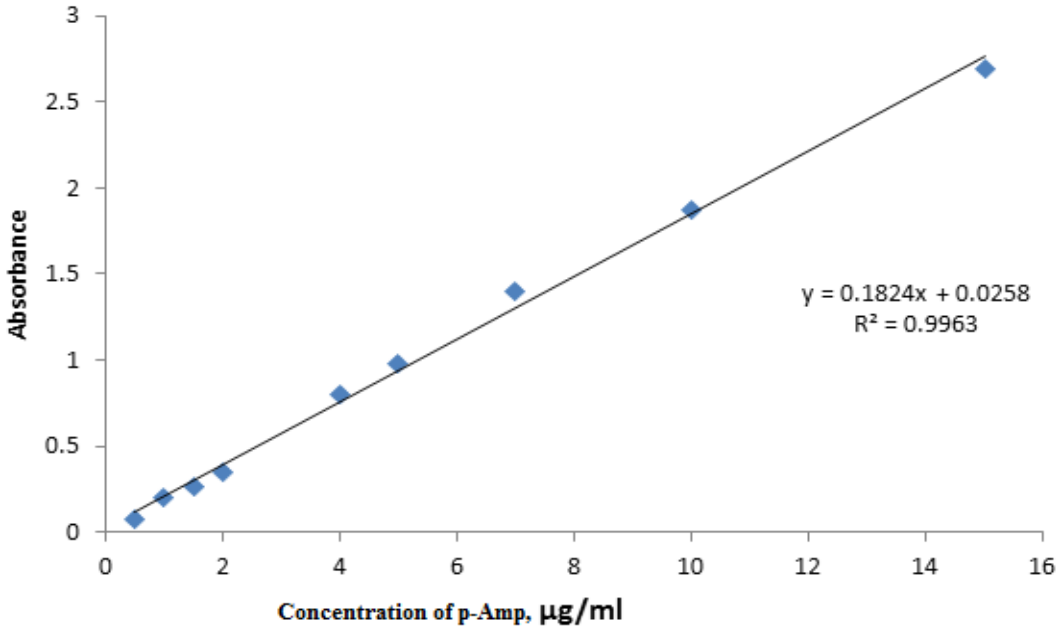


Fig. 1: The calibration curve for determination p-Amp via the suggested method.

Preliminary Study

The absorption spectrum of the colored product formed as a result of the coupling of the diazotised p-aminophenol (D-p-Amp) with 4-Chlr was studied. From adding 0.5 ml of $NaNO_2$ (1%) to 5µg of p-Amp and 0.5 ml of hydrochloric acid (1M) and left for 3 minutes and, then destroyed the remaining HNO_2 by adding 0.3 ml of sulphamic acid with 3 minutes waiting, then add 1 ml of 4-Chlr (0.1%) and 0.5 ml 1M NaOH, then with distilled water completed the volume to the mark and leaving the solution for 5 minutes at room temperature. The resulting spectrum of the orange azo dye versus the blank solution gave maximum absorption at the wavelength of 488nm., and it was fixed and used in the subsequent experiments.

RESULTS AND DISCUSSION

The determination of p-Amp, subsequent experiments were carried out using 1 ml of 50 µg/ml p-Amp solution in a final volume of 10 ml, and the absorbance of the solution was measured at the wavelength of 488 nm against the blank solution.

Optimal Conditions

Choosing the acid used in the diazotisation

Several acids used in the diazotisation reaction were studied, and through the results listed in (Table 1), it was found that hydrochloric acid is the best it gave the highest absorbance for this, it was used in subsequent experiments.

Table 1: Chose the type of acid used in the diazotization

Acid used (1M)	Absorbance	λ max (nm)	Δ λ*	Absorbance /min. standing time		
				Immediately	10	30
HCl	0.667	488	187	0.665	0.667	0.664
H_2SO_4	0.638	489	188	0.634	0.638	0.625
CH_3COOH	0.587	488	187	0.585	0587	0.574

*Color contrast.

The effect of volume of hydrochloric acid on the intensity of azo dye has been studied, the results show that 0.6 ml of 1MHCl gives the highest absorbance of the formed azo dye. Therefore, the volume was fixed in subsequent experiments.

The Effect of Sodium Nitrite and Standing Time

The effect of sodium nitrite amount was studied, by adding various volumes of sodium nitrite from 0.05 to 0.5 ml and flasks were lifted for a different period time before adding sulphamic acid and the results are listed in (Table 2).

Table 2: Effect of the amount of sodium nitrite and the time of diazotisation.

ml of 1%$NaNO_2$ solution	Absorbance / minute standing time					
	0	1	2	3	4	5
0.05	0.885	0.892	0.875	0.865	0.842	0.805
0.1	0.907	0.919	0.915	0.909	0.865	0.825
0.3	0.713	0.717	0.703	0.625	0.618	0.607
0.5	0.710	0.712	0.715	0.725	0.621	0.610

The results in (Table 2) show that the optimal volume of sodium nitrate is 0.1 ml and it needs 1 minute as a time required to complete the formation of the diazonium salt.

Studying the Effect of Sulphamic Acid and Standing Time

The unreacted HNO_2 is undesirable according to side reactions, so that it must be removed via adding increased volumes of (0.2-0.4) ml of 3%sulphamic acid (Othman and Sultan, 2014), the results illustrated in (Table 3).

Table 3: Effect of the amount of sulphamic acid and time

ml of 3% Sulphamic acid solution	Absorbance/ minute standing time				
	0	2	4	5	7
0	S=0.876	0.874	0.870	0.865	0.852
	B=0.116	0.119	0.123	0.133	0.152
0.2	S=0.921	0.927	0.866	0.823	0.759
	B=0.006	0.009	0.007	0.010	0.011
0.3	S=0.907	0.914	0.857	0.812	0.780
	B=0.009	0.005	0.008	0.010	0.020
0.4	S=0.491	0.490	0.484	0.480	0.475
	B=0.013	0.013	0.015	0.016	0.018

From the results shown in (Table 3) the optimal volume of sulphamic acid is 0.2 ml with a time of 2 minutes is enough to destroy the excess HNO_2(equation 1) and the volume and time were used in subsequent experiments.

$$HNO_2 + H_2NSO_3H \longrightarrow N_2\uparrow + H_2SO_4 + H_2O \qquad \text{…..Equation 1}$$

Effect of 4-Chlr Reagent Amount

The effect of the amount of coupling reagent (0.1% 4-Chlr) was studied, different volumes from 0.5 to 2.5ml were added and with different amounts of p-Amp from 1-15 µg/ml and the results as shown in Table 4.

Table 4: Effect the amount of 4-Chlr reagent on the absorbance of the azo dye

ml of Coupling agent (0.1%)	Absorbance/µg of p- aminophenol present / ml						R^2
	1	2.5	5	10	12.5	15	
0.5	0.150	0.374	0.748	1.128	1.497	1.914	0.9860
1	0.185	0.462	0.925	1.473	2.063	2.178	0.9830
1.5	0.183	0.458	0.916	1.549	2.073	2.169	0.9860
2	0.190	0.476	0.952	1.667	2.083	2.500	0.9980
2.5	0.171	0.428	0.856	1.326	1.958	1.752	0.9400

According to the results in (Table 4) a volume of 2 ml of the reagent 4-Chlr was chosen. This volume gave the highest value of the absorbance of the formed azo dye and the highest value of the determination coefficient.

The Effect of the Base Type

The effect of different types of the base on the adsorption of azo dye has been studied. The obtained results are illustrated in (Table 5).

Table 5: Choosing the type of the base

Type of Base used (1M)	Absorbance	pH
NaOH	0.954	12.30
Na_2CO_3	0.855	9.78
$NaHCO_3$	0.243	7.6

The results listed above showed that the reaction needs a strong alkaline medium and sodium hydroxide is giving the colored product with the highest absorbance. Also, the volume of sodium hydroxide solution has been studied. The results showed that 1 ml of 1M NaOH gave the highest absorbance, and therefore it was used in subsequent experiments.

Solvent Effect

The effect of solvents of different polarity on the absorption spectrum of azo dye was studied by dilution with various organic reagents and compared with water Fig. (2).

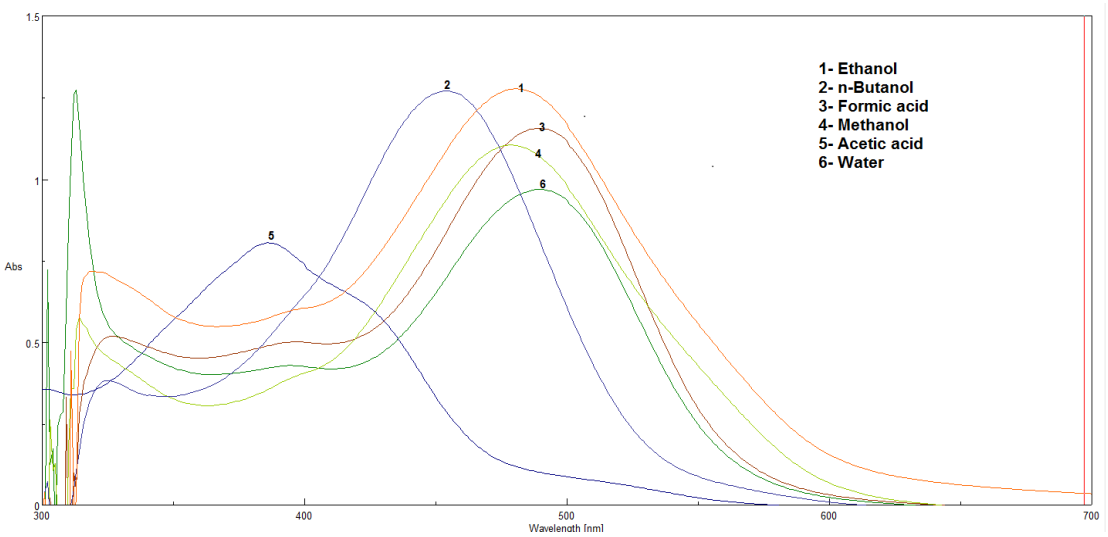


Fig. 2: Spectrum of the azo dye using different dilution solvents.

The results cited in Fig. (2) show that the organic solvents ethanol, methanol, normal butanol, and formic acid give higher absorption than distilled water, but due to the economic advantage, the availability of water, and its non-toxicity, water is recommended for dilution in subsequent experiments.

The Effect of Time on the Stability of the Formed Azo Dye

The effect of time on the absorbance of azo dye was studied by taking two different concentrations. 2.5 and 5 µg/ml and the absorption were observed every 5 or 10 minutes for 60 minutes (Table 6).

Table 6: The effect of time on the stability of the azo dye

µg of p-Amp/ml	Absorbance / minute standing time								
	0	5	10	15	25	30	40	50	60
2.5	0.434	0.435	0.436	0.436	0.437	0.437	0.435	0.435	0.426
5	0.964	0.975	0.976	0.978	0.978	0.978	0.976	0.972	0.971

From the results shown in (Table 6), it is clear that the orang azo dye was stable for at least 60 minutes.

Final Absorption Spectrum

After creating the optimal conditions shown in (Table 7), the absorption spectrum of the formed azo dye that gives a higher color absorption at wavelength 488 nm vs. the blank solution that gives very small absorption at the maximum wavelength of the measurement and 488 nm was fixed in the subsequent experiments Fig. (3).

Table 7: Optimal conditions for the proposed method

Variable	Optimality
$NaNO_2$, %, ml	1,0.1
HCl, M, ml	1, 0.6
Sulphamic acid, %, ml	3, 0.2
Reagent 4-chlororesorcinol ml, %	2, 0.1
NaOH,M,ml	1, 1

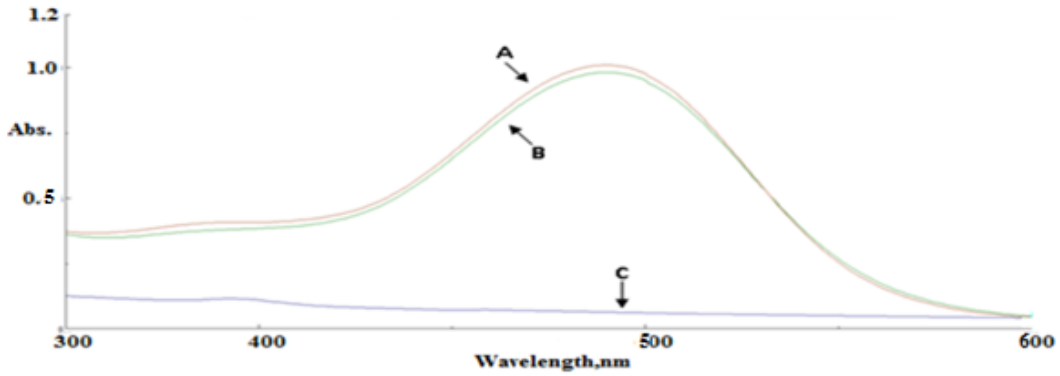


Fig. 3: Absorption spectra: (A)Colored azo dye (resulted from 5 µg p-Amp/ml) versus distilled water; (B) Colored product of azo dye versus blank solution and (C) blank solution versus distilled water.

Study the Nature of the Formed Colored Azo Dye

The methods of molar ratio and the continuous variation (Job,1970) were applied. For the study of the proportion of the composition of the orang azo dye Fig.(4A and B).

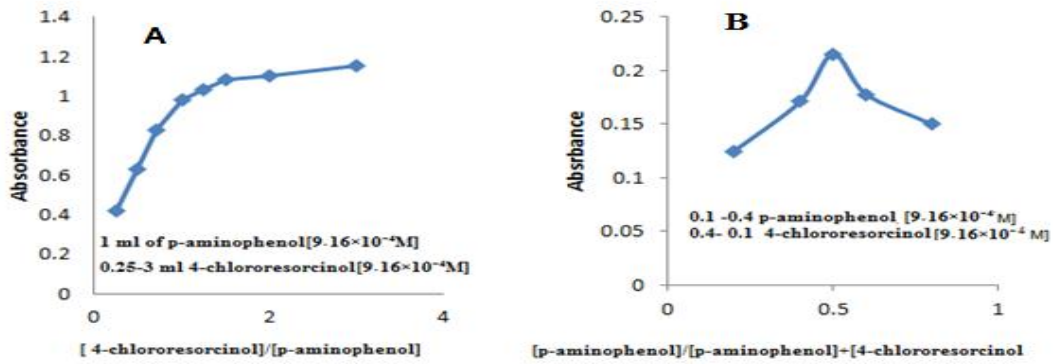
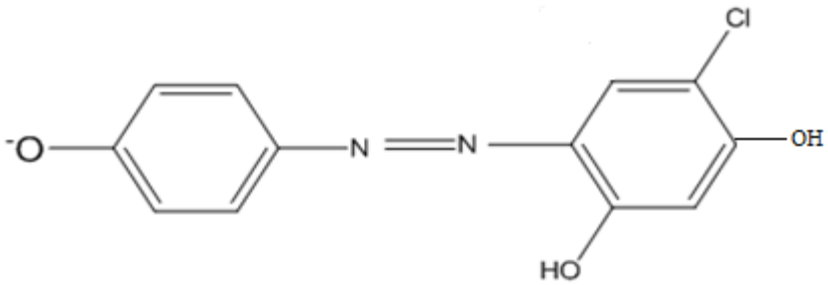


Fig. 4: (A) Plot of mole ratio method, (B) Plot of the continuous variation method

The results as shown in Fig. (4) indicated that p-Amp-4-Chlr azo dye is in a 1:1 molar ratio, and the suggest formula for the formed azo dye is shown in (Scheme 2).



Scheme 2: The suggested structure of the orange azo dye

Application of the Proposed Method

The proposed method for the determination of p-Amp has been applied in the estimation of paracetamol after acid hydrolysis to p-Amp:

Application of the method using the equation of a straight line to find the concentration of paracetamol in tablet and injection formulations (Table 8).

Table 8: Application of the proposed method for the estimation of paracetamol in pharmaceutical preparations.

Pharmaceutical preparation	Certified Value (mg)	Amount present (µg/ml)	Recovery (%)	Drug content found (mg)
Paracetamol injection	600 mg/5ml	2	99.42	596.52
		5	100.40	602.4
		10	99.83	598.98
Paracetamol tablet	500 mg/tablet	2	99.13	495.65
		5	100.30	501.5
		10	99.78	498.9

The result in (Table 8) indicates the success of the method in the determination of paracetamol in tablet and injection formulations.

Estimation by Standard Addition Method

To prove that the proposed method is free from interference, the standard addition method was applied the obtained results shown in Fig. (5 and 6) and (Table 9) indicate that the standard addition method is in good agreement with the direct method within the acceptable range of error, which indicates that the method is satisfactory and free interferences.

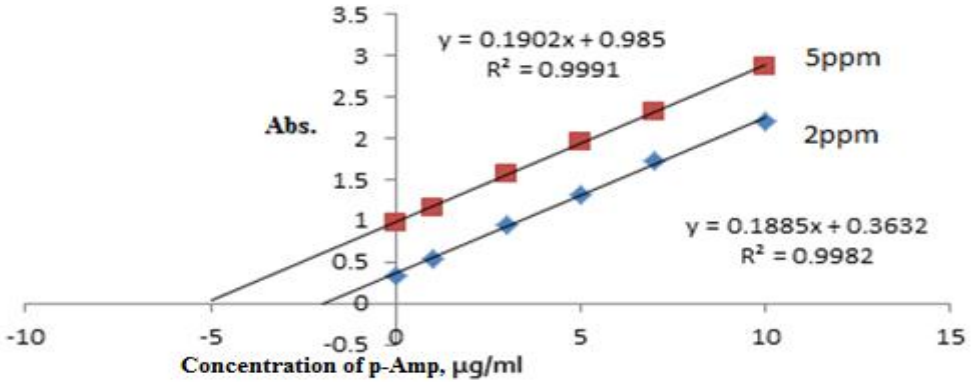


Fig. 5: Plot of standard addition method for the determination of paracetamol in injection.

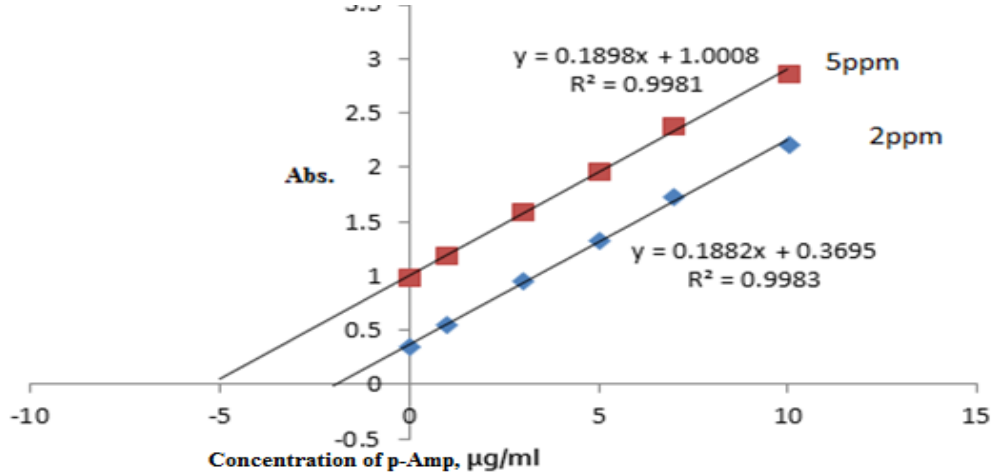


Fig. 6: Plot of standard addition method for the determination of paracetamol in tablet.

Table 9: Results of standard addition method

Drug	Amount taken	Amount measured	Recovery
Paracetamol injection	2	1.926	96.30
	5	5.178	103.56
Paracetamol tablet	2	1.963	98.15
	5	5.272	105.45

From the results shown in (Table 9), we conclude that the proposed method has proven successful and its reliability in the estimation of paracetamol after the procedure of acid hydrolysis to p-aminophenol.

Comparison with other Methods

A comparison has been made of the most important analytical variables of the currently proposed method with its counterparts in another method, as shown in (Table 10).

Table 10: Ccomparing some of the important analytical changes of the method with other methods

Variable	Present method	(Iorhemen *et al*., 2017)
Type of reaction	Diazotization and coupling	Diazotization and coupling
Reagent used	4-chlororesorcinol	1-Naphthol
max, nmλ	488	505
Linearity range(µg/ml)	0.5-15	2 – 10
Molar absorptivity $l.mol^{-1}\cdot cm^{-1}$	$1.9905x10^4$	$1.6973\ x10^4$
LOD, µg/ml	0.016	0. 020
LOQ, µg/ml	0.054	0 0. 10

From the results listed in (Table 10), we note that the proposed method is more sensitive than the method used in comparison.

CONCLUSION

An accurate spectrophotometric method has been suggested for the determination of p-aminophenol. The method is based on the diazotisation reaction of p-Amp and coupling the Diazotised p-Amp with 4-chloroisorcinol reagent in an alkaline medium. The method was applied for the indirect determination of paracetamol in tablet and injection formulations.

ACKNOWLEDGEMENTS

We extend our thanks and appreciation to the Presidency of the Department of Chemistry/ College of Science/ University of Mosul for facilitating conducting research in the Analytical Research Laboratory.

REFERENCES

Abbas, R.; Allawi, A.; Abdulhassan, N.; Mahmoud, N. (2020). Spectrophotometric determination of paracetamol using a newly synthesized chromogenic reagent 4- [(2-amino-1, 3thiazol-4-yl) amino] nitro benzene. *Egypt. J. Chemistry*, **63**(12), 4681-93.

Al-Sabha, T.; Al-Gubouri, Z. (2020). Indirect spectrophotometric determination of paracetamol via decolorization of Eriochrom Black-T With N-bromosuccinimide. *J. Edu. and Sci.*, **29**(3), 246-56.

Alubaidy, G.; Basheer, A.; Saied, S.; Thanoon, E. (2019). Spectrophotometric determination of paracetamol using diazotization coupling reaction. *Raf. J. Sci*, **28**(2),76-83.

Bloomfield, A. (2002). A sensitive and rapid assay for 4-aminophenol in paracetamol drug and tablet formulation, by flow injection analysis with spectrophotometric detection. *Talanta*, **58**(6),1301-10.

British Pharmacopeia on CD-ROM. System Simulation Ltd, the Stationary Office Ltd, London, 2009.

Crevar, M.; Ivkovic, B.; Vladimirov, S.; Kuntic, V.; Vujic Z. (2008). Statistical optimization of reverse phase high performance liquid chromatography for the analysis of caffeine paracetamol and its degradation product p-aminophenol. *Acta Chim. Slov.*, **55**, 665–670.

Dejaegher, B.; Bloomfield, M.; Smeyers-Verbeke, J.; Vander Heyde, Y. (2008). Validation of a fluorimetric assay for 4-aminophenol in paracetamol formulations. *Talanta*, **75**(1), 258-265.

Iorhemen, R.; Iorhemba, A.; Sambo, R.; Nande, W. (2017). Spectrophotometric determination of paracetamol in drug formulations with 1–naphthol. *Inter. J. Advan. Chem.*, **5**(2), 86-90.

Jamal, M.; Sarac, A.; Magner, E. (2004). Conductive copolymer-modified carbon fiber microelectrodes: electrode characterization and electrochemical detection of p-aminophenol. *Sens. and Act. B: Chem.*, **97**(1), 59-66.

Job, P. (1971). "Spectrochemical Methods of Analysis". Wiley Intersience, New York, 346 p.

Khosroshahi, A.M.; Aflaki, F.; Saemiyan, N.; Abdollahpour, A.; Asgharian, R. (2016). Simultaneous determination of paracetamol, 4-aminophenol, 4-chloroacetanilid, benzyl alcohol, benzaldehyde and EDTA by HPLC method in paracetamol injection ampoule. *J. Pharm. and Heal. Sci.*, **4**(1), 61-69.

Mahmood, H. (2020). A friendly environment approach for determination of paracetamol. *J. Edu. and Sci.*, **29**(2), 230-40.

Mitchell, S.C.; Waring, R.H. (2002). "Aminophenols." In Ullmann's Encyclopedia of Industrial Chemistry; 2002 Wiley-VCH

Mohamed, M.; Abdelakder, H.; Abdellah, B. (2021). Microwave assisted synthesis of 4-aminophenol Schiff bases: DFT computations, QSAR/Drug-likeness proprieties and antibacterial screening. *J. Mol. Str.*, **1241**, 130666.

Othman, N.S.; Sultan, S.H. (2014). Spectrophotometric determination of niclosamide by prior reduction and subsequent diazotization-coupling with 2,6-dihydroxybenzoic acid – Application to tablets. *Raf. J. Sci*., **25**(1), 40-52.

Othman, N.S.; Zakaria, R.A. (2009). Spectrophotometric determination of p-aminophenol via oxidative coupling reaction with N-(1-naphthyl) ethylendiamine dihydrochloride in presence of potassium iodate– Application to paracetamol. *Nat. J. Chem.*, **34**, 247-259.

Othman, N.S.; Zakaria, S.A. (2007). Spectrophotometric assay of paracetamol in pharmaceutical preparations. *J. Edu. and Sci.*, **19**(3), 21-32.

Šatínský, D.; Brabcová, I.; Maroušková, A.; Chocholouš, P.; Solich, P. (2013). Green chromatography separation of analytes of greatly differing properties using a polyethylene glycol stationary phase and a low-toxic water-based mobile phase. *Anal. and Bio. Chem.*, **405**(18), 6105-15.

Singh, M.; Sahu, A.; Mahata, S.; Singh, P.; Rai, V.; Rai, A. (2019). Efficient electrochemical determination of p-aminophenol using a novel tri component graphene-based nanocomposite. *New J. Chem.*, **43**(37), 14972-14978.

Younis, M.S.; Othman N. S. (2019). Rifampicin as a novel reagent in spectrophotometric assay of paracetamol. *Eur. J. Anal. Chem.*, **14**(4),19-24.

Younis, M.S.; Othman, N.S. (2018). Indirect spectrophotometric assay of paracetamol in pharmaceutical preparations. *Inter. J. Enh. Res. in Med. and Den. Care (IJERMDC)*, **5**(4), 23-29.

Younis, M.S.; Othman, N.S. (2018). Using of 2,5-dihydroxy benzaldehyde in spectrophotometric assay of paracetamol in pharmaceutical preparations. *Inter. J. Rec. Res. and Rev.*, **XI**(2), 10-17.

تقدير البارا أمينوفينول عن طريق الآزوتة والاقتران مع 4- كلوروريسورسينول – التطبيق على الباراسيتامول

الملخص

تم اقتراح طريقة طيفية لتقدير البارا– امينو فينول بصوره مباشرة. الطريقة تتضمن اجراء تفاعل أزوتة للبارا– امينو فينول باستخدام زيادة من نتريت الصوديوم وبوجود حامض الهيدروكلوريك وبعد تحطيم المتبقي من حامض النتروزو بإضافة حامض السلفاميك ثم الاقتران مع الكاشف 4– كلوروريسورسينول بالوسط القاعدي من هيدروكسيد الصوديوم لتكوين صبغة ازوية ملونة ذائبة بالماء تعطي اعلى امتصاص عند الطول الموجي الاعظم 488 نانوميتر. المدى الخطي الذي يتبع قانون بير كان من 0.5 الى 15 مايكرو غرام / مل. وبلغت قيمة معامل الامتصاص المولاري $1.9905x10^4$ لتر/مول. سم. بعد تثبيت الظروف المثلى وذلك بدراسة جميع العوامل المؤثرة على امتصاص الناتج المتكون تم تطبيق الطريقة في تقدير الباراسيتامول في مستحضراته الصيدلانية بعد اجراء تحلل مائي حامضي وتكوين بارا –امينو فينول وكانت النتائج على درجة عالية من الدقة وضمن الاخطاء المقبولة تحليليا وتم تطبيق طريقة الاضافة القياسية والنتائج اثبتت عدم وجود تداخل من قبل المضافات.

الكلمات الدالة: بارا– امينو فينول، تفاعل الآزوته الاقتران، 4– كلوروريسورسينول و باراسيتامول.