Eurasian Journal of Analytical Chemistry
ISSN: 1306-3057 OPEN ACCESS 2019 14 (4): 19-24

Rifampicin as a Novel Reagent in Spectrophotometric Assay of Paracetamol

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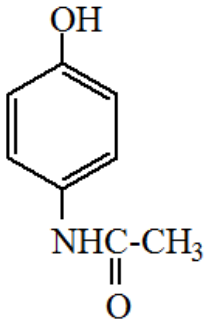
Received: 16 July 2019 ▪ Revised: 17 August 2019 ▪ Accepted: 18 September 2019

Abstract: Rifampicin (REF), an antibiotic, is used for the first time to assay paracetamol in bulk sample and in its dosage forms. The method is based on adding a known excess of N-bromosuccinimide oxidant to the p- aminophenol, resulting from acidic hydrolysis of paracetamol. The residual oxidant is allowed to react with a fixed amount of Rifampicin reagent and the absorbance of the resulting colored solution is measured at 475 nm(λ_{max} of REF). The absorbances measured are found to increase linearly with the amount of paracetamol present originally in solution, Beer's law is obeyed over the concentration of 2.5 to 15 µg/ml paracetamol with a determination coefficient (R^2) of 0.9979,molar absorptivity factor of 4.63x 10^3 $l.mol^{-1}.cm^{-1}$,Sandell's sensitivity index of 0.033 $\mu g.cm^{-2}$. The quality control/assurance analytical variables such as limits of detection (LOD), limits of quantification (LOQ) are also reported. The method is used successfully to assay paracetamol in its pharmaceutical dosage form (tablets, syrup and injection).

Keywords: Paracetamol Assay, Rifampicin, Novel Reagent, N-bromosuccinimide, Color Bleaching.

INTRODUCTION

Paracetamol (PAR) [also called acetaminophen, N-acetyl-*p*-aminophenol, 4-acetamidophenol], is used as a common analgesic and antipyretic drug that is used for the relief of fever, headaches and other minor aches and pains[1]. PAR is available in different pharmaceutical preparations such as: tablet, drops, capsules, injection and syrup [2]. PAR has the following chemical structure [3].



PAR , M.Wt = 151.2 g/mol

The literature survey reveals that various methods for quantitative determination of PAR, these methods included: HPLC[4], HPTLC [5], RP-HPLC [6,7], Voltametry [8-11], and other electrochemical[12].

Spectrophotometric methods have taken the functional groups present in PAR and in reduced-PAR and used in its determination [13-21]. Rifampicin(REF), also known as rifampin, is an antibiotic used to treat several types of bacterial infection The solid form of the compound is a crystalline brownish-red powder [3]. To the best of our knowledge, this compound has not yet been used as analytical reagent to any extent and this manuscript is devoted to the analytical use of this compound in the assay of PAR in its pharmaceutical preparations.

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The method is simply based on the oxidation of p-aminophenol, resulting from the acidic hydrolysis of PAR, with a known excess of N- bromosuccinimide oxidant and the residual of the later is allowed to react and bleach the color of affixed amount of REF chromogenic reagent.

EXPERIMENTAL

Instruments

All spectrophotometric measurements are performed on Jasco V-630 Spectrophotometer using 1 cm quartz cells.

Reagents

The chemicals used in present investigation are of analytical – reagent grade, and PAR standard material was provided from General Establishment for Medical Appliance and Drugs / SDI – Samarra / Iraq.

1) Hydrolysed Paracetamol Solution, 100 µg.ml^{-1}

This solution is prepared by transferring 150 ml of 1000 µg.ml^{-1} PAR into 250 ml round-bottomed flask provided with condenser, 25ml of 11.8N hydrochloric acid is then added and the mixture is refluxed for 1 hour .After cooling the solution is neutralized with 20% of sodium carbonate solution and diluted to the mark in a 250 ml volumetric flask with distilled water. To prepare a solution equivalent to 100 µg.ml^{-1} PAR, (16.6) ml of the neutralized solution is diluted to 100 ml in a volumetric flask using distilled water(Younis, and Othman,2018).

2) PAR Tablets Solution, 100 µg.ml^{-1}

A 10 tablets (each one contains 500 mg PAR) are weighed and finely powdered .Then an accurately weighed portion of the fine powder, equivalent to 0.25g PAR is dissolved in 10 ml ethanol, then 100-150 ml distilled water are added with shaking to enhance the solubility, The resulting solution is filtered into 250 ml volumetric flask and the solution is completed to the mark with a distilled water and procedure is then followed as in the preparation under hydrolysed PAR solution.

3) PAR Syrup Solution, 100 µg.ml^{-1}

A 10.41 ml of antipyrol syrup(each 5ml contain 120 mg PAR) is diluted to 250 ml with distilled water in a volumetric flask, then 150 ml are taken and procedure as mentioned above is followed.

4) PAR Injection Solution, 100 µg.ml^{-1}

The contents of 3 injections (each 1ml contain 100 mg PAR) are thoroughly mixed and 2.5 ml is diluted to 250 ml with distilled water in a volumetric flask. Then 150 ml of the resulting solution is taken and procedure mentioned above is followed.

5) Other Solutions

Other solutions, 1x10^{-3}M N- bromosuccinimide (NBS) and 1.5x10^{-3} M REF are prepared by dissolving the accurate weight in the corresponding solution volumes .1M of HCl is prepared by dilution of concentrated acid solution with distilled water.

RECOMMENDED PROCEDURE

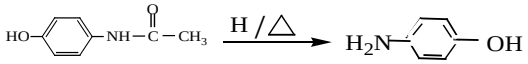
Aliquots of hydrolysed PAR solution was transferred into a series of 10 ml calibrated flasks to cover the range of concentration from 25 to 150.Then 0.5 ml of 1M of HCl and 1 ml of 1x10^{-3} M of NBS oxidant solution are added and the reaction mixture is settled for 5 min. at room temp. (23˚C). 1ml of 1.5 x10^{-3}M of REF solution is added then the flasks are diluted to the mark with distilled water . After 10 min. the absorbance at 475 nm (wavelength of maximum absorption of REF) versus the reagent blank is measured.

RESULTS AND DISCUSSION

Principle of the Method

The method involves four steps:

Hydrolysis of PAR in acidic medium to p- amino- phenol(PAP) .



Paracetamol p-Aminophenol

2-Oxidation of p- aminophenol by excess NBS.

PAP+ NBS (excess) → PAP(oxidized product) +NBS(unreacted)

3-Residual NBS oxidizes fixed amount of REF and bleaches color.

NBS (unreacted) + REF → Oxidized product of REF + Unreacted REF(indicator bleaching)

4- The REF remaining after step 3 is measured at 475 nm.

Study of the Optimum Reaction Conditions

During the investigation, 100 μg hydrolysed PAR solution are taken and final reaction mixture volumes are 10 ml. All factors affecting and related to the colored reaction mixture have been studied and optimum condition are selected.

1) REF Reagent

The optimum amount of the REF reagent that an spectrophotometrically determined is first examined. Fig. 1 shows that up to 1ml of 1.5 x10 $^{-3}$ M of REF (dye) solution satisfies Beer's law and hence is selected.

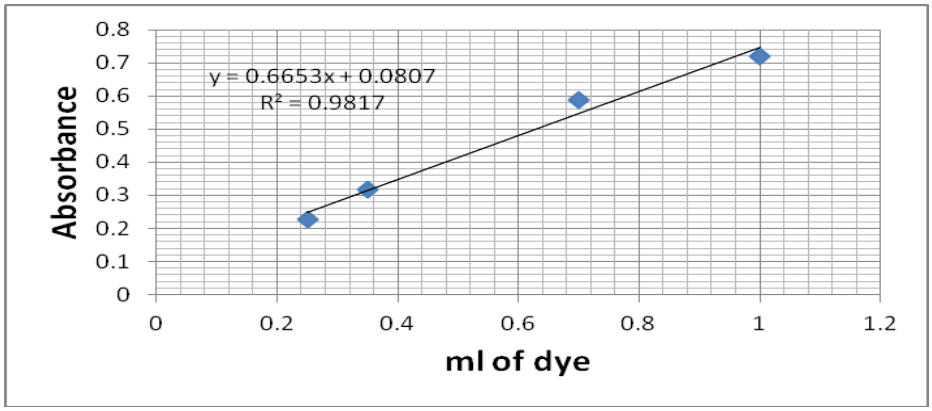


Fig. 1: The optimum amount of REF

2) Choice of Oxidant

Various oxidizing agents(KIO_4 , NBS and N- chlorosuccinimide) have been tested for bleaching the color of REF(experiments are without hydrolysed PAR), NBS gives maximum bleaching(Fig. 2).

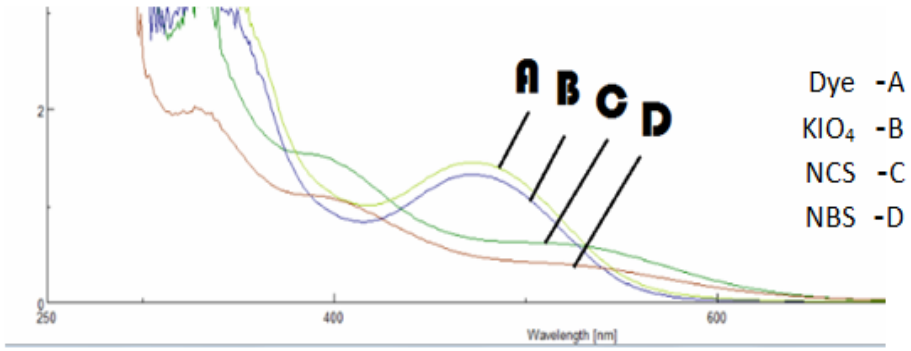


Fig. 2: The effect of oxidant in bleaching the color of REF

NBS has been selected for subsequent experiments because of its stronger oxidizing properties compared to KIO_4 and N- chlorosuccinimide. It may be attributed to the stronger oxidising properties of bromonium ion as compared to chloronium ion in acidic medium.

3) Effect of NBS Amount

The experimental results obtained revealed that 1.0 ml of 1x10^{-3} M NBS solution gives the optimum analytical performance. Therefore, this amount has been selected for the subsequent experiments.

4) Medium of Reactions

Acidic medium is found to be a favorite one for both oxidation and color bleaching. 0.5 ml of 1M HCl is found suitable for both steps.

5) Effect of Time on Oxidation and Bleaching

The experimental data have been shown that the time factor plays an important role in both oxidation of hydrolysed PAR and bleaching of REF chromogenic reagent. The results are given in Table 1. A 5. minutes oxidation time and 10 minutes bleaching time are recommended for subsequent work. Longer times result in a lower intensity due probably to side reactions.

Table 1: Effect of time on oxidation and bleaching

Standing time of oxidation (minute)	A/ Standing time before dilution (minute)				
	5	10	15	20	30
5	0.305	0.309	0.310	0.304	0.301
10	0.238	0239	0.233	0.232	0.231
15	0.230	0230	0.234	0.237	0.231
20	0.217	0219	0.218	0.213	0.212

Calibration Curve

Under the above established optimum conditions, a linear relationship is obtained between the absorbance and the concentration of PAR within the range 25-150 µg.10 ml^{-1} (2.5-15 $\mu g.ml^{-1}$) and a concentration above 15 $\mu g.ml^{-1}$gives a positive deviation (Fig. 3).

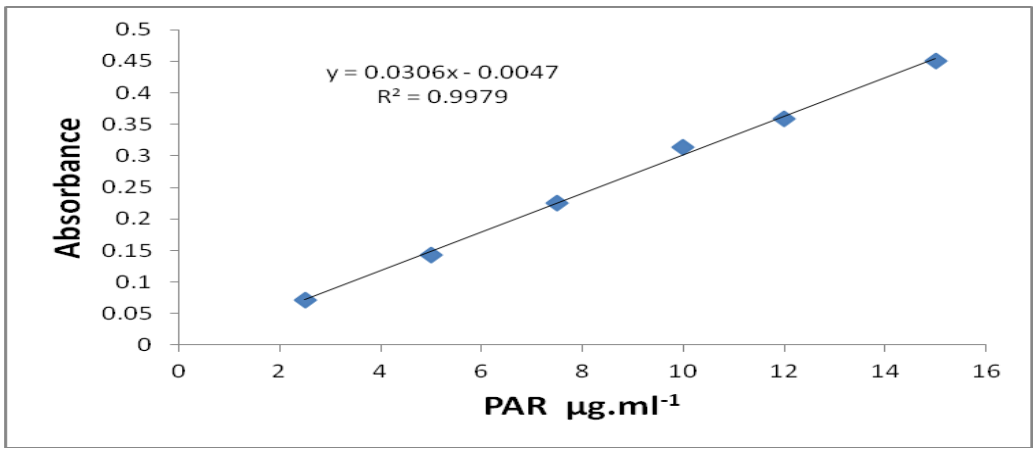


Fig. 3: The calibration graph for PAR determination

The molar absorptivity factor, Sandell's sensitivity index, LOD and LOQ are given in Table 2, revealing a high sensitivity of the present method.

Table 2: Optical and regression characteristics of the present method.

Parameter Value
Beer's law($\mu g.ml^{-1}$) range 2.5 - 15
λ max 475
Molar absorpitivity l .mol. $^{-1}$ cm^{-1} 4.625 $x10^3$
Relative Standard Deviation ≤ ± 0.36
Linear regression equation Y= a x -b Slope = a 0.0306 Intercept = b -0.0047 X= Concentration in $\mu g.ml^{-1}$
Determination coefficient (R^2) 0.9979
LOD (100 $\mu g.ml^{-1}$) 0.7302
LOQ (100 $\mu g.ml^{-1}$) 2.434

***Average of five determinations.**

Analytical Application

The proposed method has been shown to be successful in estimating PAR in pharmaceutical preparations. The results in Table 3 indicate that there has a good accuracy (RE%) and precision(RSD%).

Table 3: Results of determination of hydrolysed PAR in formulations

Pharmaceutical preparation	µg **PAP** Present/10ml	**µg** PAP **measure**	Rec.*%	%RE*	RSD%*
Paracetamol injection 500 mg / 5 ml India	50	49.2	98.4	-1.6	1.7
	100	101.7	101.7	1.7	2.1
Antipyrol-syrup 120mg SDI/Iraq	50	48.3	96.6	1.4	1.2
	100	101.7	101.7	1.7	0.36
Paracetamol 500mg/tablets	50	48.7	97.4	-2.4	2.9
	100	99.2	99.2	0.8	1.4

***Average of five determinations.**

The results are also compared statistically by a Student's t- test for accuracy with those of the standard method[3] at 95 %confidence level. The calculated t- value(for tablets =2.07 and for injection=2.04) did not exceed the tabulated value (2.306) for eight degrees of freedom indicating that there is no significant difference between the proposed method and the standard method.

CONCLUSION

The suggested procedure for PAR determination is sensitive, accurate and can be used in determination PAR in different types of formulations without extraction or separation. The most important advantage in this work is the possibility of using REF in spectrophotometric method to estimate the compound under study (PAR). Therefore, REF can be used in the future in estimating many pharmaceutical compounds.

REFERENCES

[1] Bosch, M.E., Sánchez, A.R., Rojas, F.S., & Ojeda, C.B. (2006). Determination of paracetamol: Historical evolution. *Journal of pharmaceutical and biomedical analysis*, *42*(3), 291-321.

[2] Martindale, S.S. (1999). *The complete drug reference.* 36th Edn. Pharmaceutical press, London.

[3] British Pharmacopeia on CD-ROM, 3rd Edn. System Simulation Ltd, the stationary office, London, (2007).

[4] 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. *Journal of Pharmaceutical and Health Sciences*, *4*(1), 61-69.

[5] Abdelaleem, E.A., Naguib, I.A., Hassan, E.S., & Ali, N.W. (2015). HPTLC and RP-HPLC methods for simultaneous determination of paracetamol and pamabrom in presence of their potential impurities. *Journal of pharmaceutical and biomedical analysis*, *114*, 22-27.

[6] Topkafa, M., Ayyildiz, H.F., Memon, F.N., & Kara, H. (2016). New potential humic acid stationary phase toward drug components: Development of a chemometric-assisted RP-HPLC method for the determination of paracetamol and caffeine in tablet formulations. *Journal of separation science*, *39*(13), 2451-2458.

[7] Thomas, S.P., & Poomali, A. (2016). Development and Validation of RP-HPLC Method for Simultaneous Estimation of Paracetamol and Tramadol Hydrochloride. *Indian Journal of Pharmacy and Pharmacology*, *3*(4), 169-172.

[8] Kalambate, P.K., Sanghavi, B.J., Karna, S.P., & Srivastava, A.K. (2015). Simultaneous voltammetric determination of paracetamol and domperidone based on a graphene/platinum nanoparticles/nafion composite modified glassy carbon electrode. *Sensors and Actuators B: Chemical*, *213*, 285-294.

[9] Yilmaz, S., Bas, Z., Sadikoglu, M., Yagmur, S., & Saglikoglu, G. (2016). Sensitive voltammetric determination of paracetamol on poly (4-Aminobenzene Sulfonic Acid) modified glassy carbon electrode. *Int. J. Electrochem. Sci.*, *11*, 6244-6255.

[10] Sadok, I., & Tyszczuk-Rotko, K. (2015). New, Simple and Sensitive Voltammetric Procedure for Determination of Paracetamol in Pharmaceutical Formulations. *Insights Anal Electrochem*, *1*, 1.

[11] Lima, A.B., Torres, L.M., Guimarães, C.F., Verly, R.M., Silva, L.M.D., Carvalho Júnior, Á.D., & Dos Santos, W.T. (2014). Simultaneous determination of paracetamol and ibuprofen in pharmaceutical samples by differential pulse voltammetry using a boron-doped diamond electrode. *Journal of the Brazilian Chemical Society*, *25*(3), 478-483.

[12] Wang, H., Zhang, S., Li, S., & Qu, J. (2018). Electrochemical sensor based on palladium-reduced graphene oxide modified with gold nanoparticles for simultaneous determination of acetaminophen and 4-aminophenol. *Talanta*, *178*, 188-194.

[13] Younis, M.S., & Othman, N.S. Indirect Spectrophotometric Assay of Paracetamol in Pharmaceutical Preparations. *International Journal of Enhanced Research in Medicines and Dental Care*, *5*(4), 23.

[14] Omar, F.K. (2014). Indirect spectrophotometric determination of paracetamol in different pharmaceutical samples. *Iraqi National Journal of Chemistry*, (53), 36-42.

[15] Iorhemen, R.T., Iorhemba, A.M., Sambo, R., & Nande, W.U. (2017). Spectrophotometric determination of paracetamol in drug formulations with 1–naphthol.. *International Journal of Advanced Chemistry*, *5*(2), 86- 90.

[16] Al-Shwaiyat, E.A. (2013). Spectrophotometric determination of paracetamol by reduction of 18-molybdo-2-Phosphate heteropoly anion. *Jordan Journal of Chemistry*, *8*(2), 79.

[17] Saeed, A.M. (2017). Spectrophotometric determination of paracetamol in some manufactured tablets in Iraqi markets. *Int J Pharm Sci Rev Res*, *42*(2), 53-57.

[18] Lawrence, O.A., Olufemi, A.G., Alex, O.D., & Kayode, S.T. (2012). Spectrophotometric determination of acetaminophen content of different brands of paracetamol tablets from South-West Nigeria *Journal Research in Environmental Science and Toxicology*, *1*(10), 251.

[19] Vichare, V., Mujgond, P., Tambe, V., & Dhole, S.N. (2010). Simultaneous spectrophotometric determination of paracetamol and caffeine in tablet formulation. *International Journal of Pharmaceutical Technical Research*, *2*(4), 2512-2516.

[20] Behera, S., Ghanty, S., Ahmad, F., Santra, S., & Banerjee, S. (2012). UV-visible spectrophotometric method development and validation of assay of paracetamol tablet formulation. *J Anal Bioanal Techniques*, *3*(6), 151-157.

[21] Delvadiya, K., Kimbahune, R., Kabra, P.K.S., & Patel, P. (2011). Spectrophotometric simultaneous analysis of paracetamol, propyphenazone and caffeine in tablet dosage forms. *Int J Pharm Pharm Sci*., *3*(3), 170-74.