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A Green Indirect Spectrophotometric Estimation of the Analgesic Naproxen in the Existence of the Another Analgesic Paracetamol

Thura Zeyad Al-Sarraj [a]*, Ammar Masood Al-Taee [b]

[a]*Department of Pharmacy, Alnoor University College, Bartella, Iraq*
[b]*Department of Dental Industry Techniques, Alnoor University College, Bartella, Iraq*

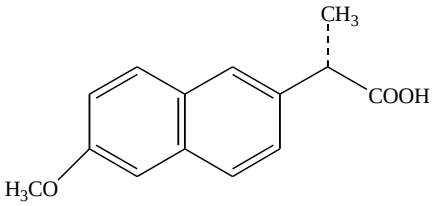
Abstract

A novel and secure indirect spectrophotometric technique for estimation of naproxen as a coupled compound with paracetamol as a unharmed diazotized reagent than organic reagents. Naproxen was first converted into an active form to act as coupling agent by reflux with hydrobromic acid (HBr) and acetic acid (CH_3COOH) to yield the Hydroxy Form of Naproxen (HFN). The diazotized complex absorb light in wavelength 500 nm. Beer's law linearity is 10 to 200 µg/20ml, (i.e. 0.5-10.0 ppm), the molar absorptivity (ε) is $2.14x10^4$ l/mol.cm, Sandell's sensitivity index (SSI) is 0.0094 µg/cm which indicate a high sensitivity, RSD more than ±0.36 % indicate good precision and higher accuracy (relative error more than 0.82 %), and LOD = 0.024 µg/ml and LOQ = 0.079 µg/ml. The technique has been used effectively for estimation of naproxen in tablets.

Key words: Naproxen; Novel; Paracetamol; Diazonium; Indirect.

1. Introduction

Naproxen is possessing chemical formula ($C_{14}H_{14}O_3$ (230.259 g/mol)) and physical properties is a white, crystalline powder, melting point is 152° to 154°, insoluble in water and soluble in ethanol, chloroform, and ether [1]. The chemical structure was shown as below [2].



Naproxen

As the solubility of naproxen is poor some hydrotropic solvent used in spectrophotometric estimation of naproxen to overcome the hazardous influences of organic co-solvent [3].

The hydroxy analogous of the naproxen acts as a coupling agent for estimation of paracetamol, in which the hydrolysis product of paracetamol was act as a diazotizing agent to form red color absorb the visible light at 500 nm. The method is utilized for the estimation of paracetamol in presence of naproxen [4].

The oxidation of HNF is performed by potassium permanganate ($KMnO_4$) in two procedures, in the first one, in acidic medium, the decrease of absorbance $KMnO_4$ is proportional with increase in HFN at 545 nm. In the second, in basic medium, ($KMnO_4$) was used to oxidize the HNF; the absorbance of manganate produced from reaction is 610 nm. Both methods have been effectively utilized for the determining drug in tablets form [5]. 2,2 bipyridyl was used in estimation of HNF after oxidizing it by Fe^{+3} and complexometric with 2,2 bipyridyl in the water bath 35° C [6].

Other techniques for quantitative estimation of Naproxen are including Chemiluminescence [7,8] or they based on electrochemical method [9], and Ion selective electrodes [10,11], HPLC procedures [12,13] in addition to spectrophotometric methods in ulta violet region [14,15]. A colorimetric estimation of naproxen depends on chemical derivatization with reagent 4-carboxyl-2,6-dinitrobenzene diazonium ion [16].

Naproxen was estimation by formulation of ion-pair with bromophenol blue to form complex [17,18], The second procedure of ion pair based on acidic

*Corresponding author e-mail: thura.zayad@alnoor.edu.iq
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reaction of two sulfophthale dyes with naproxen, first one is bromocresol green and another is bromothymol blue [19].

The p-aminobenzoic acid diazotization is used as a reagent to estimation of HNF in sodium hydroxide medium. Then extraction the active ingredient of naoroxen from **tablets** by ethyl acetate [20].

The aim of this work is the estimation of naproxen as a modified coupling agent in the presence of hydrolyzed paracetamol (diazotized p-aminophenol) as a safer compound than chemical reagents, friendly environment, and less toxic to researchers.

2. Experimental

2.1. Apparatus

1- Spectrophotometer Jasco V-630 double-beam with 1.0 cm quartz cells.

2- pH meter HANNA 301 used to pH measurements.

3- For weight measurements, was used BEL balance

4- Electro thermal Wisd stirrer is utilized as heater.

2.2. Reagents

All chemicals substances utilized were high analytical grade.

Synthesis of hydroxy form of Naproxen (HFN),0.04 mol of fresh Naproxen (the weight required is 9.2 g) mixed with (48%) hydrobromic acid (25 ml) and (25 ml) of concentrated acetic acid, then transferred this mixture in reflux for a time (1.5 hour) and then cooled in room temperature, added 25 ml distilled water, filtrate, desiccation and recrystallization
using ethanol to make a pink solid crystal having melting point (190-191 C°) [21].

HFN (100 µg / ml), It was make by dissolve 0.01g of naproxen (SDI) in 2 ml of organic solvent (ethanol) and transferred in the volumetric flask then completed to 100 ml using distilled water. The solution put in brown bottle and is remain stable in one month.

Sodium Nitrite ($NaNO_2$) 1%, is prepared by dissolving 1.0 g from pure salt in distilled water and the volume was filled to 100 ml.

Hydrochloric acid (HCl 1M), is prepared by dilution concentrated Hydrochloric acid (11.6M) 8.6 ml in 100 ml distilled water .

Sodium hydroxide solution (NaOH 1M), is prepared by convenient dilution of concentration solution to 1000 ml distilled water.

Extraction from tablet,10 tablets of pure naproxen was refine to a very small particles and a weight equal to one tablet ,dissolute into ethyl acetate (3 ml) and one ml HCl (3M), then separated into two layer after separation, then organic layer was carried to another tube and extraction was recurrent 3 times, added 1 ml of saturated solution of sodium chloride to organic layer and sufficient quantity of sodium sulphate . The layer was quit on air for evaporating solvent [22].

Paracetamol solution PAR (1000 µg/ml): is prepared by dissolving 0.25 g of pure paracetamol in 10 ml ethanol then the solution was fulled to 250 ml with distilled water

Solution of hydrolyzed paracetamol (HPAR): is prepared by transferring 150 ml of 1000 µg/ml PAR into 250 ml round bottomed flask contain 25 ml of concentrated hydrochloric acid, then refluxed for 1 hour, after that cooling solution was neutralized with 20% of sodium carbonate solution and diluted to 250 ml with distilled water [23] .

p-Aminophenol ($1x10^{-3}$ M), is prepared by 25 ml of concentrated HPAR solution dilution to 100 ml by using distilled water.

2.3. Experimental part

Step-1- Synthesis of HFN.
Step-2- preparation of HPAR.
Step-3- Formation of diazonium salt.
Step-4- Formation of colored azo product.

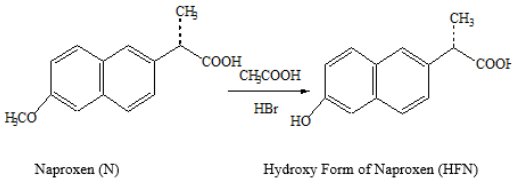

Fig.1. Synthesis of HFN

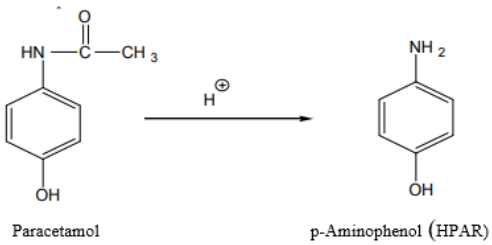

Fig.2. Hydolysis of paracetamol

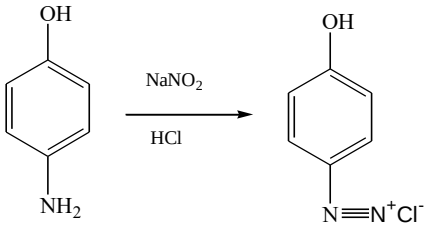

Fig 3. Formation o.f diazonium salt

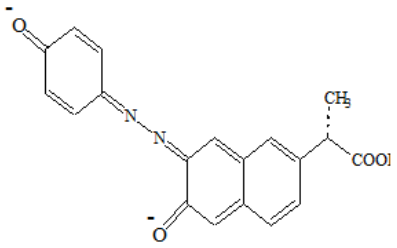

Fig.4. Formation of colored azo product

2.4. Study the optimum conditions of reaction:

2.4.1. Select type of acid and its quantity

We study the influence of different quantity of many acids on absorption in Table 1

From Table (1), H_3PO_4 (1M) in the volume (1.5 ml) was selected for the following experiments as the best absorptions intensity of the colored complex.

Table 1: Acid selection and its quantity

Acids (1M)	Absorbance per ml of acid				
	0.5	1.0	1.5	2.0	2.5
HCl	0.124	0.227	0.312	0.122	0.069
H_2SO_4	0.170	0.152	0.143	0.146	0.136
HNO_3	0.171	0.170	0.145	0.116	0.121
H_3PO_4	0.294	0.439	0.520	0.095	0.060
CH_3COOH	0.163	0.234	0.333	0.516	0.162

2.4.2. Study the quantity of nitrite with time

from (0.1 ml to 1.2 ml) of $NaNO_2$ (1%) has been checked with a pause time from (0 to 5) min, the yields are listed in Table (2)

From Table (2), 0.5 ml of 1% $NaNO_2$ has been chosen with one minute as favorable time for reaction.

2.4.3. Sulphamic acid quantity with time

We added between (0.3-1.5) ml of 3% of sulphamic acid solution at different pausing time with shaking the absorbance was measured.

From Table (3), the best reaction time is 1 min when added 0.5 ml of sulphamic acid (3%)

Table 2: Nitrite quantity with time

Volumes in ml of (1%) $NaNO_2$ solution	Absorbance per minute of pausing time				
	1	2	3	4	5
0.1	0.535	0.536	0.546	0.547	0.546
0.3	0.548	0.538	0.543	0.544	0.545
0.5	0.550	0.546	0.544	0.542	0.543
0.7	0.542	0.543	0.542	0.542	0.542
1.0	0.549	0.544	0.544	0.543	0.542
1.2	0.548	0.543	0.543	0.546	0.548

Table 3: Influence of sulphamic acid quantity with time

ml of sulphamic acid (3%) solution	Absorbance/minute , pause time with a shake				
	1	2	3	5	7
0.3	0.083	0.037	0.038	0.038	0.039
0.5	0.552	0.547	0.547	0.546	0.542
0.7	0.549	0.550	0.547	0.548	0.446
1.0	0.546	0.548	0.550	0.549	0.548
1.2	0.545	0.546	0.549	0.546	0.548
1.5	0.545	0.549	0.548	0.546	0.549

2.4.4. Influence of diazotized agent quantity

Influence of (1-5) ml of (1×10^{-3}) M diazotized HPAR against (10-150) µg/20 ml of HFN. Table (4) appears 2 ml of diazotized HPAR solution gives the favorable yield.

Table 4.
Influence of diazotized agent quantity

ml of Para (1×10^{-3}) M	Absorbance/µg of Nap						R^2
	10	30	50	70	100	150	
1	0.066	0.162	0.271	0.386	0.542	0.628	0.9993
2	0.075	0.172	0.288	0.392	0.548	0.826	0.9995
3	0.075	0.156	0.267	0.378	0.547	0.816	0.9969
4	0.085	0.141	0.243	0.357	0.534	0.789	0.9955
5	0.052	0.136	0.234	0.350	0.529	0.773	0.9948

2.4.5. Selection of base and its quantity

Two bases NaOH, and KOH and other two basic salts (Na_2CO_3, and $NaHCO_3$) at different volumes (1ml to 5ml) of each solution to the influence on the absorption of the dye produced. The yields are listed in Table (5).

From Table (5) we have chosen 2 ml of sodium hydroxide

Table 5: Selection of base and its quantity

Type of base (1M)	ml of base and base salt				
	1	2	3	4	5
NaOH	0.172	0.545	0.488	0.337	0.292
KOH	0.144	0.328	0.428	0.329	0.218
Na_2CO_3	0.023	0.028	0.242	0.546	0.541
$NaHCO_3$	0.044	0.444	0.361	0.235	0.175

2.4.6. Influence of surfactant

So as to study the influence of surfactants on absorption, 2ml of [Sodium dodecyl sulphate] (SDS), [Cetylpyridinium chloride] (CPC), [Cetyltrimethyl ammonium bromide] (CTAB) surfactants in different sequence of additions was illustrative in Table (6).

Table 6: Influence of surfactants

(1×10^{-3}M) of Surfactant solution	Absorbance/sequence** of addition					
	I	II	III	IV	V	VI
SDS	0.507	0.510	0.522	0.535	0.545	0.543
CTAB	0.415	0.431	0.446	0.445	0.462	0.464
CPC	0.413	0.415	0.427	0.438	0.436	0.434

*Absorbance without addition of surfactant = 0.545(sequence I)
** I. (R) HPAR +(S) surfactant + (H) H_3PO_4 + (N) $NaNO_2$+ (A) Sulphamic acid + (D) HFN+ (B) NaOH
II. HPARl (R) +H+S+N+F+D+B
III. HPAR (R) +H+N+S+A+D+B
IV. HPAR (R) +H+N+A+S+D+B
V. HPAR (R) +H+N+A+D+S+B
VI. HPAR (R) +H+N+A+D+B+S

2.5. Absorption spectrum

A noticing and measuring of the azo colored product exhibit it stays stable at least 60 min. The figure below (Fig. 5) shows the absorption curve of the product formed after adaptation of the conditions of the reaction.

2.6. Calibration curve and procedure

(0.1-2.0) ml of 100 µg/ml standard HFN solution was added without cooling depend on the following sequence and quantities:

2ml of HPAR (1 x 10^{-3} M), 1.5 ml of (H_3PO_4) ,0.5 ml of $NaNO_2$ (1%), 0.5 ml of sulphamic acid (3%), HFN, and 2 ml of (1M) NaOH has been finally added, the distilled water was added to complete the volume to 20 ml in the volumetric flask, the absorbance has been estimated at 500 nm. Figure 6.

We can notice in the range (10-200 µg) of HFN, (0.5-10 ppm) the calibration curve is linear, ε = 2.4 x 10^4 l/mol.cm, SSI is 0.0094 µg/cm^2, LOD = 0.024 µg/ml and LOQ = 0.079 µg/ml.

2.7. Accuracy and precision

To examination the accuracy and precision of the calibration curve, at three various concentrations the HFN is determined and the yields are shown in Table (8), which mention perfect accuracy and precision.

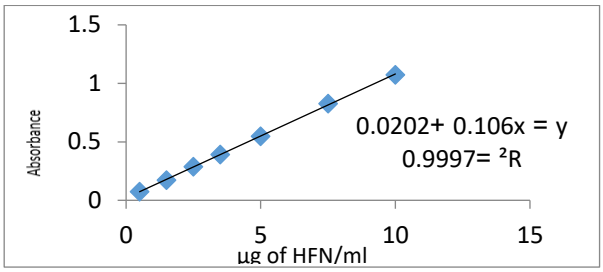

Fig.6. The calibration curve of spectrophotometric method

Table 8: accuracy and precision

Quantity HFN taken µg/20 ml	RE %*	RSD %*
50	0.12	±0.29
100	-0.41	±0.36
150	1.35	±0.21

*Average of four measurements.

2.8. Nature of the azo product

We can be using two methods to study the composition of azo dye, first is a job's method and other is a mole ratio method. The yield show that 1:1 azo dye is the propose structure as in figure 4.

2.9. Stability constant (K)

The stability constant of the azo dye in aqueous solution is found 2.4x10^4 l/mol. The yields of estimation are illustrated in Table (9).

Table 9: (K) Stability constant

ml of HFN (1×10^{-3} M)	Absorbance			K^* (l/mol)
	A_s	A_m	α	
0.1	0.023	0.031	0.359	5.0x10^4
0.2	0.123	0.170	0.382	2.1x10^4
0.5	0.153	0.263	0.712	0.11x10^4

K^* (l/mol) = 2.403x10^4

2.10. Influence of organic solvents

In the Fig. 7 illustrate the using of acetic acid (CH_3COOH) and water (H_2O) is more detectable, while other solvents are causing turbidity or separated into two-layer. H_2O is still the best solvent. Figure 7. show the influence of solvents

2.11. Influence of interferences

In order to illustrate the analytical application of this teqniuqe , we have been added 100 µg of HFN in the existence 100 ,300, 500,1000 µg of another compounds that expected presence in the drug. The yield is shown in Table (10).

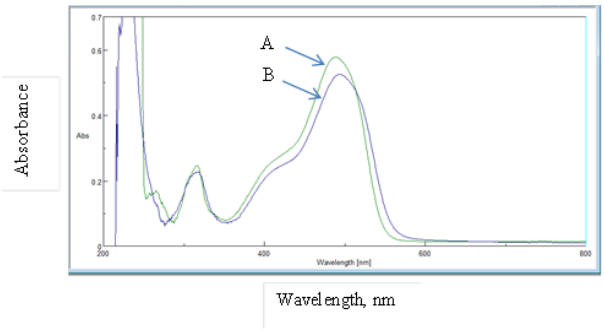

Fig.7. Influence of solvents

Table 10: Influence of interferences

Interferences	% of Recovery 100 µg HFN /µg of Interferences			
	100	300	500	1000
Starch	100.0	101.6	102.7	103.2
Glucose	0.991	102.2	101.2	100.3
Gum Arabic	97.1	100.2	101.0	100.4
Lactose	98.9	97.2	99.45	102.1

2.12. Application of the technique

To test the check of the applicability the present technique, it has been utilized to the estimation of naproxen in the tablets. The yields are showed in Table (11) illustrate the best applicability of the technique.

Table 11: Application of the technique

Quantity of HFN /20 ml	Recovery (%) of NAP*		
	Naproxen (tablet 500 mg) - Bilim	Naproxen (tablet 250 mg) , Iraq (S.D.I)	Naproxen (tablet 500mg) - Damascus - Syria
50	103.0	102.4	101.0
100	97.0	97.2	98.7
150	99.4	97.8	99.5

*Average of four estimations.

2.13. t-test

At the same time, both the current technique and British Pharmacopeia technique [24] has been utilized to t-test computation [25] and the value was compared with statistical Tables at 95% validation level for 4 degree of freedom.

Table 12
t-test

Drug	Recovery * %		t-exp
	Current technique	British Pharmacopeia technique	
(Tablet of naproxen 500 mg)- Bilim	99.8	99.9	0.055
(Tablet of naproxen 500mg) - Damascus ,Syria	99.7	97.4	0.25

* Average of four estimations.

The yield in Table (12) show that there is no actual difference between these techniques.

3. References

[1] Karch A. M., 2010, Lippincott's Nursing Drug Guide, Lippincott Williams and Wilkins, New York, 834.

[2] Acomoffat O. MD. and Widdop B. 2005. Clarke's Analysis of Drugs and Poisons ", CD ROM.

[3] Maheshwari R. K., Lakkadwala S., Vyas R. and Ghode P.,2010. Spectrophotometric determination of naproxen tablets using niacinamide as hydrotropic solubilizing additive, J. Current Pharm. Res., 4, 11-14.

[4] Mahmood H. Sh., 2020. A Friendly Environment Approach for determination of paracetamol", J. Edu.Sci., 29(2)
doi: 10.33899/edusj.2019.125964.1011.

[5] Mahmood H. Sh., and Al-Sarraj T. Z., 2020. Two novel spectrophotometric methods for determination of naproxen via a modulation to hydroxy analog, Current, Appl. Sci. Tech., 20(2), 295-309.
.https://doi:li01.tcithaijo.org/index.php/cast/article/view/241041/164826.

[6] Mahmood H. Sh., and Fatih-Alla T. Z. 2020. A novel approach for determination of naproxen, Inter. J. Pharm. Res., 12(2),1473-1480.

[7] Yinhuan Li., Jiuru Lu., 2006. Flow injection chemiluminescence determination of naproxen based on $KMnO_4$-Na_2CO_3 reaction in neutral aqueous medium, Analytica Chemica Acta , 577(1),107-110.

[8] Campiglio A., 1998. Determination of naproxen with chemiluminescence detection, Analyst, 123, 1571-1574.

[9] Soltani N., Tavakkoli N., Mosavimanesh Z. S., Davar F., 2018. Electrochemical determination of naproxen in the presence of acetaminophen using acarbon paste electrode modified with activated carbon nanoparticles, Comptes Rendus Chimie, 21(1), 54-60.

[10] Lenik J., Dumkiewick R., Wardak C., B., Marczewska ,2002. Naproxen ion-selective electrode and its application to pharmaceutical analysis, Acta Poloniae pharmaceutica-drug research ,59(3)171-176.

[11] Valsami G. N., Macheras P. E., Koupparis M. A., 1989. Construction of a naproxen ion-selective electrode and its application to pharmaceutical analysis, Analyst, 3.

[12] Wahbi AM., Mabrouk M., Moneeb M., Kamal A., 2009. Simultaneous determination of the two non-steroidal anti-inflammatory drugs; diflunisal and naproxen in their tablets by chemometric spectrophotometry and HPLC, Pak. J. Pharm. Sci., 22(1) 8-17.

[13] Mikami E., Goto T., Ohno T., Matsumoto H., Nishida M., 2009. Simultaneous analysis of naproxen, nabumetone and its major metabolite 6-methoxy -2-naphthylacetic acid in pharmaceuticals and human urine by high performance liquid chromatography, J. Pharm. Biomed. Anal., 23(5) 917-925.

[14] Nijhu R. S., Jhanker Y. M., Sutradhar K. B., 2011. Development of an assay method for simultaneous determination of ciprofloxacin and naproxen by uv spectrophotometric method, Stamford J. Pharm. Sci., 4(1), 84-90.

[15] Sloka S. N., Upadhyay B. M., Kumar Ch. A.,2011. Simultaneous Spectrophotometric determination of

naproxen and Pantoprazole in pharmaceuticals dosage form, J. of Applied Chem. Res., 17, 65-74.

[16] Jain N. A., Lohiya R. T, Umekar M. J., 2011. Spectrophotometric determination of naproxen and esomeprazole in a laboratory mixture by Simultaneous equation, absorption correction, absorption ratio and area under curve methods, Inter. J. Pharma. Sci. and Res., 2(5), 130-134.

[17] Idowu S. O., Adegoke O. A., Adeniji A. O., Olaniyi A. A. ,2009. Colorimetric Assay of naproxen tablets by derivatization using 4-carbonyl-2,6-dinitrobenzene diazonium ion, East and Central African Journal of pharmaceutical sciences, 12(1), 8-14.

[18] Keyhanian F., Alizadeh N., Shojaie A. F., 2014. Spectrophotometric determination of naproxenas ion –pair with bromophenol blue in bulk, pharmaceutical human serum samples, Current Chemistry Letters, 3, 15-22.

[19] Alizadeh N., Keyhanian F., 2015. Simple, sensitive and selective spectrophotometric assay of naproxen in pure, pharmaceutical preparation and human serum samples", Acta Poloniae pharmaceutica-drug research, 72(5), 867-875.

[20] Mahmood H. Sh., and Al-Sarraj T.Z., 2019. A creative indirect spectophotometric determination of naproxen". Rafidain Journal of Science. 28(1), 51-60.
doi: 10.33899/rjs.2019.159393

[21] Al-Hamdany F. K., 2009. Synthesis of some (s)-2-(6'-methoxy-2'- naphthyl) propanoic acid derivatives (Naproxen), Ph. D. Thesis, Mosul University , 39,62.

[22] WWW.cp.edu,chem319=isolation of naproxen ;/files/classes=/424/lab info and exps Ka Le;proje.

[23] Dixit R. B. and Patel J. A., 2007. Spectrophotometric determination of paracetamol drug using 8-hydroxyquinoline, international journal of pharmaceutical sciences and research, 19(3),21-32.
https://doi.org/10.33899/edusj.2007.51321.

[24] British Pharmacopeia. 7th Edn., System Simulation, Ltd the Stationary Office, London… , CDROM ,2013.

[25] Christian G. D., 2004. Analytical Chemistry ,6th Edn., John Wiley and Sons, Inc., New York , 90.