Determination of Naproxen in Pharmaceutical preparations by spectrophotometric and flow Injection – activated chemiluminescence methods

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Accepted: 2011/2/8, Received: 2010/2/17

Abstract

This study involves development of a simple spectrophotometric method and a new flow injection–activated chemiluminescence (FIA-Cl) for the determination of Naproxen (Nap) in Pharmaceutical preparations .

Spectrophotometric method was based on the oxidation of the (Nap) with alkaline potassium permanganate, the reaction is followed spectrometrically by measuring the absorbance of (Nap) at 608 nm .

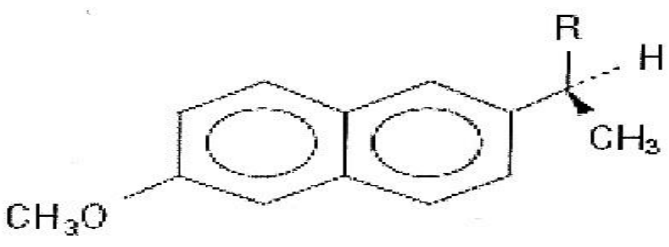
The reaction time of oxidation of (48 min) method is adopted for determining the drug concentration. The calibration graph was linear in the range of (0.4-2.8)µg.ml^{-1} with a correlation coefficient of (0.9998), detection limit of (0.281) µg.ml^{-1}, molar absorption coefficient is 2.348×10^4 L/mol.cm and a relative standard deviation RSD% of (3.12-1.32%). The method of FIA-CL was based on the activation of luminol – cobalt – H_2O_2 chemiluminescence by (Nap). The linearity is (10-45) µg.ml^{-1} with detection limit of (5.5)µg.ml^{-1}, and correlation coefficient was (0.9999) n=6 and the relative standard deviation was (1.65-1.12%).

The two methods were applied successfully to determine the content of (NaP) in pharmaceutical preparations with a recovery of 98.99%

Introduction

Naproxen (Lebbe *et al.* , 1997) is a proprionic acid derivate related to the arylacetic acid group of nonsteroidal anti-inflammatory drugs. it is antipyretic and analgesic effects were related to inhibition of cyclooxyenase.

The chemical names for naproxen and naproxen sodium (Meijer & Molema, 1995) are (S) –6– methoxy –a– methyl –2– naphthalene acetic acid and (S) –6– methoxy –∞– methyl –2– naphthalene acetic acid, Sodium salt, respectively. Naproxen and naproxen sodium have the following structures, respectively:



Naproxen (R= COOH) $C_{14}H_{14}O_3$, M.wt = 230.26

Naproxen Sodium (R=COONa) $C_{14}H_{13}NaO_3$, M.wt = 252.23

Various methods based on HPLC have been developed for determination of (Nap) as its metabolites and enatiomers in plasma, (Karidas *et al.*, 1993; Singh *et al.* 1991), and in Synovial fluid and plasma (Andersen , 1992) (Blagbrough *et al.*, 1992) and in human plasma and urine by means of gradient HPLC (Vree *et al.*, 1992) (Hansen, 1992). Also this drug has been determind in a human serum by capillary electrophoresis (Helena *et al.* , 1992) with ultraviolet absorbance and Laser – induced fluorescence detection.

Lotfi and Frida (2003) used HPLC with porous graphitic carbon (PGC) column and tetrahydro furan–methanol as the mobile phase to determine the (Nap) as degradation products (Baeyens *et al.*, 1991) used microbore liquid chromatography (LC) with native fluorescence Detection for the determination of (NaP) in pharmaceutical preparations with 0.2µg.ml^{-1} detection limit.

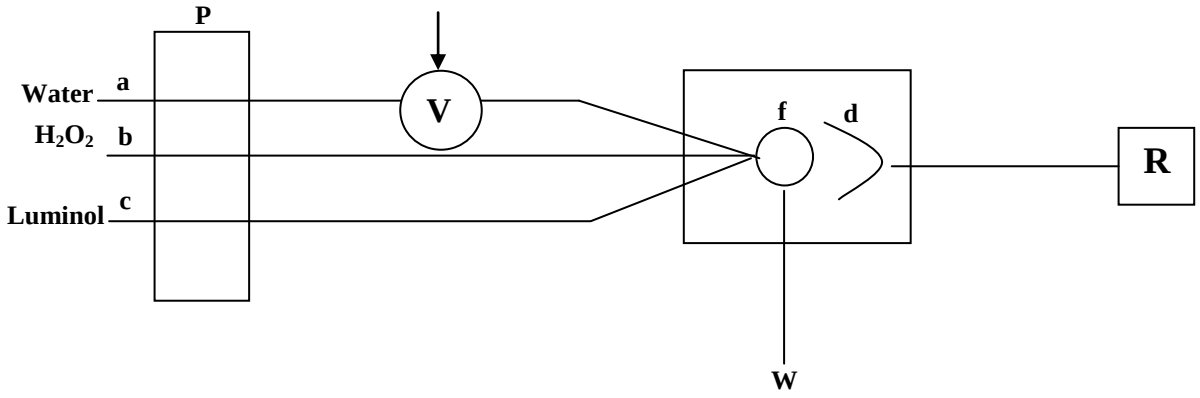
(Holzbecher, M.et al,1979) used an ultraviolet spectrophotometric procedure for the routine determination of (Nap) in serum. Also, another methods have determined (Nap) by titrimetry in tablets (Maheshwari *et al.*, 2009), Capillary electrophoresis (Fillet *et al.*, 1998), spectrofluorometry (Damiani *et al.*, 2002), flow injection analysis (FIA) using UV-detector (sener *et al.*, 2003), potentiometric titration (Hakan *et al.*, 2008), Phosphorimetric (Inmaculada *et al.*, 1999) and a rapid chemiluminescence method for the determination of (Nap) in pharmaceutical preparation based on the chemiluminescence reaction with Cerium(IV) in sulfuric acid medium(Campiglio, 1998; Hadir, 2008) used Synchronous Luminescence spectrometry to determine binary mixture of (NaP) and Difunisal.

The present paper describes a spectrophotometric method for the determination of (NaP) in pharmaceutical preparations based on the oxidation with alkaline potassium permanganate. This study also Includes development of FIA-CL method .

Experimental

A) A spectrophotometer of type Hach /USA moldel DR 4000 Uv-Visible was used, with quartz cells of 1 cm width.

B) FIA Chemiluminescence configuration which was outlined in figure (1) was used for the determination of (Nap).



P- Peristaltic pump V- Injection valve F- Flow cell d- Photomultiplier R- Recorder W- Waste solution .

Fig. (1): Chemical reaction manifold used for the Flow-Injection Chemiluminescence determination of Nap.

Reagents

Reagents of analytical grade and distilled water were used through out the study ..Solutions were prepared by appropriate dissolution as shown in table (1).

Table (1): preparation of some solutions

Substance	Molar Concentration (M)	Dissolved weight (gm)	Final Volume (ml)
N a O H	1.0	4.000	100
Na_2CO_3	0.1	10.599	1000
$Na_2C_2O_4$	0.1	13.390	1000
$KMnO_4$	0.1	15.800	1000
Luminol	1×10^{-3}	0.1772	1000

Luminol solution was prepared by dissolving the required weight in a solution of (0.1 M Na_2CO_3) where as $KMnO_4$ was prepared and standardized with 0.1M $Na_2C_2O_4$.

Hydrogen peroxide solution (1M) was prepared by diluting 45.72ml of H_2O_2 (48%) in 1 L of distilled water and standardized against standard 0.1 M $KMnO_4$.

A 0.55 ml of sulfuric acid (96%) was diluted in 100 ml of distilled water and standardized against 0.1 M Na_2CO_3. Cobalt(II) ($100\mu g.ml^{-1}$) was prepared by dissolving 0.4039 gm of $CoCl_2.6H_2O$ in 20 ml of 5×10^{-3} M H_2SO_4 and diluted to 1L with distilled water. Finally, NaP stock solution ($100\mu g.ml^{-1}$) was prepared by dissolving 0.01 gm of NaP powder in 1L of ($0.5\mu g.ml^{-1}$) cobalt (II) solution. Solutions of lower concentrations were prepared by appropriate dilution .

Pharmaceutical preparation

Naproxen Yellow tablet: provided from (SDI) Samara - Iraq. Ten tablets were taken and a certain portion of the powder was accurately weighed to give an equivalent to 500 mg of Naproxen and then dissolved with (25)ml of methanol. The resulting solution was washing by shaking with methanol and filtered on Whatman filter paper No.4 to remove any suspended particles. The filterate and washing were evaporated to dryness at $60C^0$ and the residue was redissolved in distilled water forming a solution of 100 $\mu g.ml^{-1}$ concentration .

The same method was adopted for preparation of Naproxen tablets, and the final dissolution was in (0.5 $\mu g. ml^{-1}$) cobalt and ultrasonication was needed.

Procedure (1): Spectrophotometric determination of Naproxen in pharmaceutical preparation .

General procedure for the spectrophotometric method

Initial rate method : Aliquots of 0.01M $KMnO_4$ solution (1.0 ml) and 1M NaOH solution (4 ml) were transferred into a 10ml volumetric flask . An accurate volume of the working solution of Nap (0.1– 1.0) ml was added and diluted to volume with distilled water. The contents of the mixture were shaken well and immediately transferred to the spectrophotometric cell at room temperature. The absorbance of the oxidation reaction of (Nap) was measured at 608 nm as a function of time against reagent blank. In the second procedure, the absorbance was measured at a fixed time of (48 min) and was plotted against the final concentration of Nap and the content of the drug was calculated from either the calibration graph or regression equation .

Results and Discussion

In an alkaline medium, potassium permanganate oxidizes Nap, resulting in the formation of manganate ion (Rahman & Kashif, 2003), which showed an absorption peak at 608 nm.

Because of the intensity of the color increased with time, a spectrophotometrically based method was elaborated for the determination of Nap in dosage forms. Initial parameters for this study are given in table (2).

Table (2): Initial Experimental parameters for the spectrophotometric method .

Item	Preliminary parameter	Value
1	Conc. Of $KMnO_4$	0.01 M
2	Conc. of NaOH	1.0 M
3	Volume of $KMnO_4$	1.0 ml
4	Volume of NaOH	4.0 ml
5	Reaction Time	8 min
6	WaveLength λ_{max} (nm)	608 nm

The various experimental parameters affecting the formation of the reaction product were optimized as follows :

Effect of the $KMnO_4$ concentration :

To study the effect of the $KMnO_4$ concentration , aliquots of Nap containing 10µg $.ml^{-1}$ were transferred into a series of 10ml volumetric flasks, followed by addition of varying volumes of 0.01M $KMnO_4$ (0.1-1.3) ml and 4.0 ml of 1M NaOH solution. The absorbance at 608nm was measured at a fixed time of 48 minutes. It is obvious from figure (2) that the absorbance increased with increasing volume of the $KMnO_4$ solution, and became constant at 1.1 ml and so then 1.1 ml of $KMnO_4$ was used as the optimal volume.

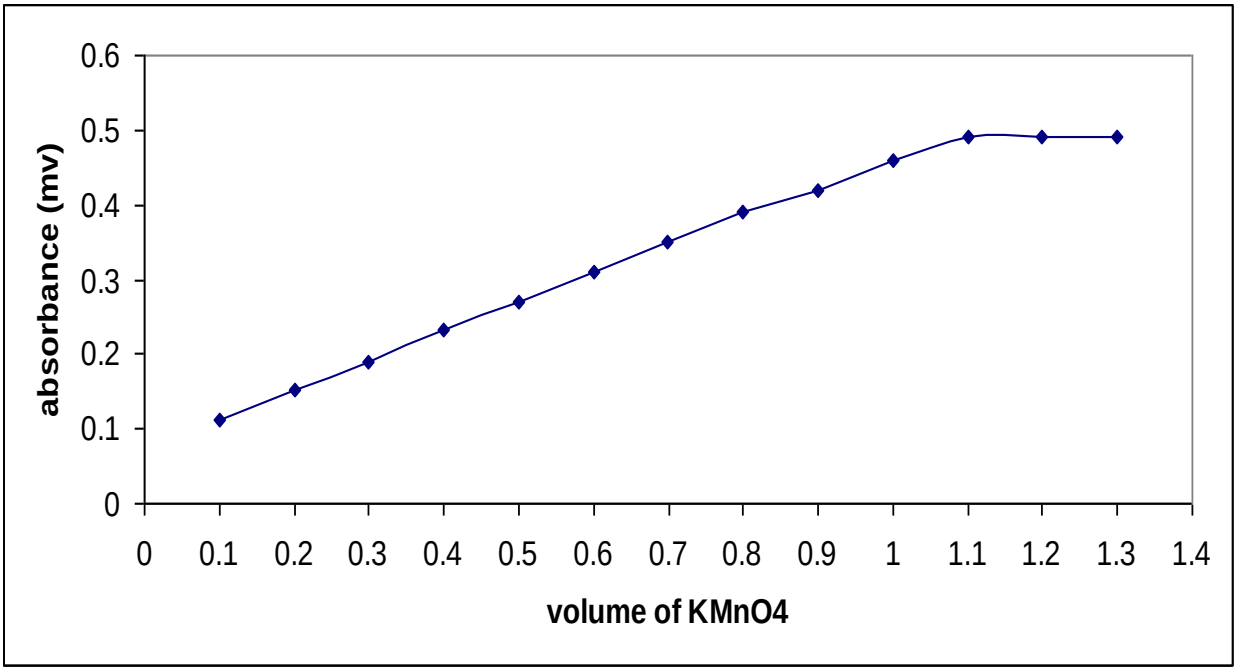


Fig. (2): Effect of the volume of 0.01M $KMnO_4$ on the intensity of color produced for the reaction (Nap 10 µ g / ml , 4.0 ml of 1 M NaOH).

Effect of the NaOH concentration

influence of the NaOH concentration on the formation of MnO_4^{2-} was examined critically. Fig.(3) shows that the maximum absorbance was obtained with 4.0 ml of the 1M NaOH, so the optimum volume of 4.0 ml was chosen.

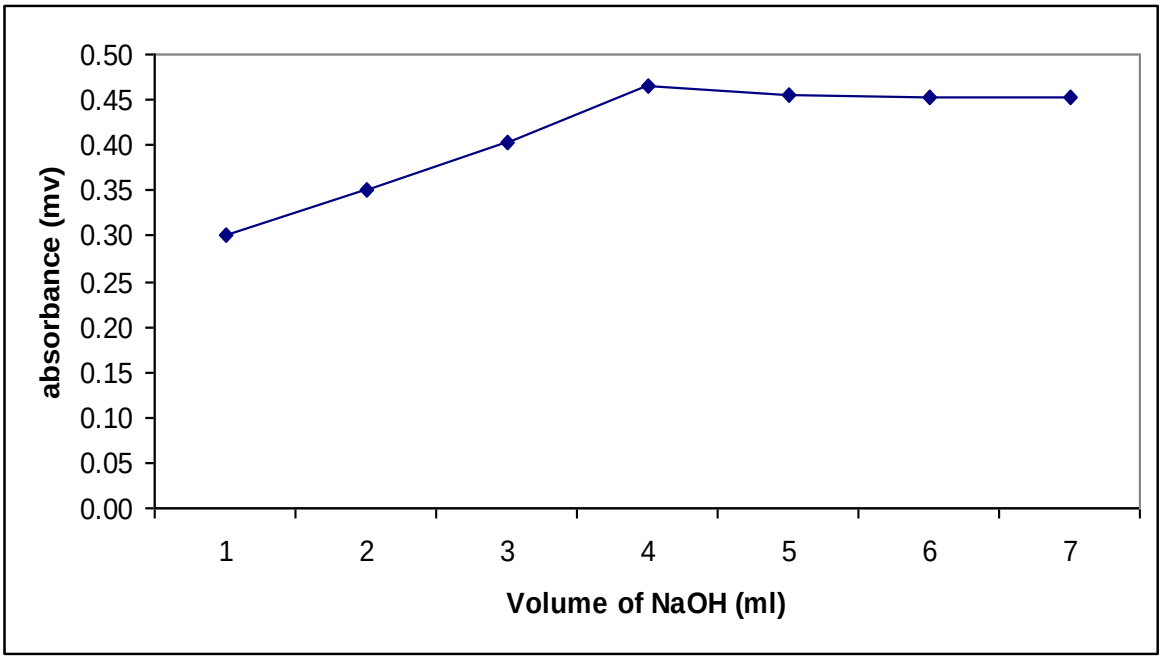


Fig. (3): Effect of the volume of 1M NaOH on the intensity of color produced for the reaction (Nap 10 µ g /ml , 1.1 ml of 0.01 M $KMnO_4$).

Effect of oxidation time

It was found that the most acceptable value was obtained at a fixed time of 48 min. Fig.(4) and therefore was considered to be the most suitable time of oxidation reaction .

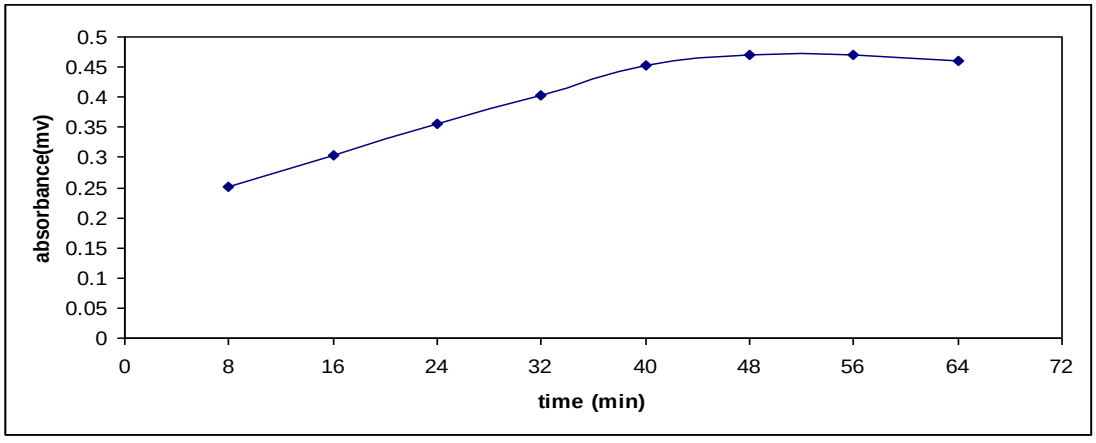


Fig. (4): Effect of time on the oxidation of Naproxene by 0.01M KMO_4.

Stability period

The stability of the oxidation product was studied through measuring the absorbance of the resulted oxidation product at different time periods. Table (3) shows the resulted product is formed immediately and is stable for more than one hour.

Table(3): stability time of oxidation reaction on the absorbance of (Nap) by spectrophotometric.

Naproxen Conc.µg/ml	Absorbance/min. standing time					
	10	20	30	40	50	60
2.0	0.432	0.436	0.433	0.439	0.440	0.438
2.4	0.514	0.523	0.519	0.524	0.531	0.529
2.8	0.601	0.608	0.604	0.610	0.609	0.607

Final absorption spectrum of (Nap)

A clear peak of MnO_4^{-2} at 608 nm fig.(5) was obtained after the oxidation of Nap by 0.01M $KMnO_4$ solution in an alkaline medium of 1M NaOH.

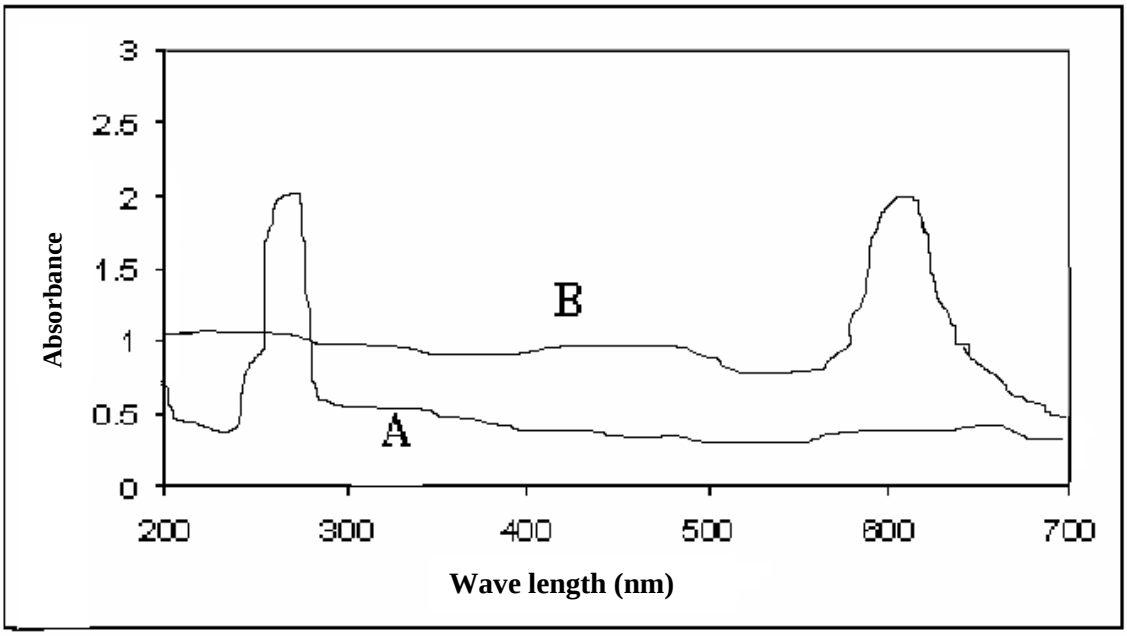
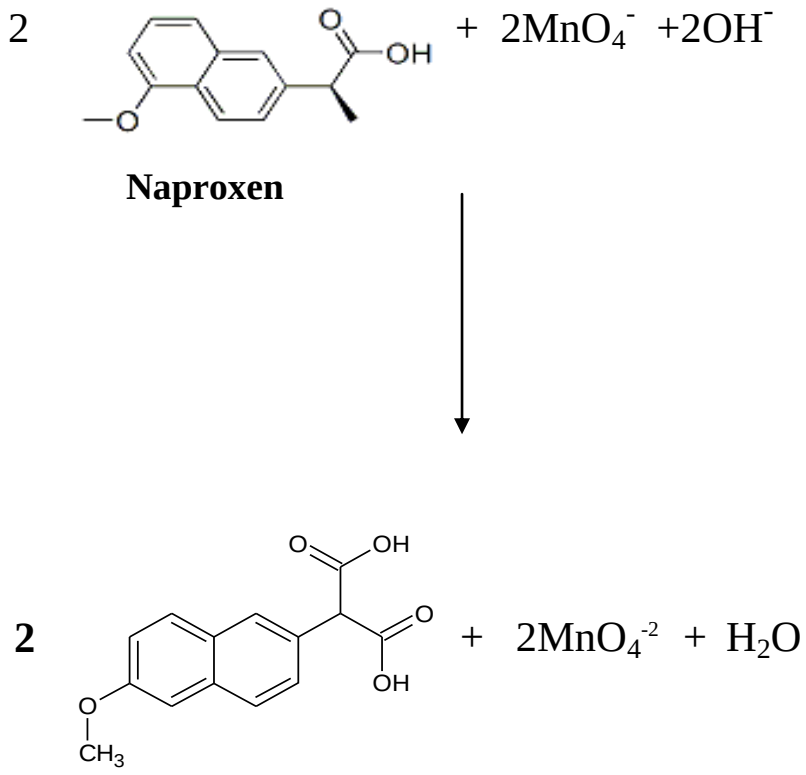


Fig. (5): A-absorption spectrum of (Nap) before oxidation with blank solution B-The absorption after the oxidation of (Nap) with $KMnO_4$ in an alkaline medium.

Naproxen contains methyl group which has an ability to be oxidized. The reaction mechanism is proposed and given in the following equations. (Raman, 2003)



Recommended analytical conditions

According to the obtained results, the optimum conditions for the determination of Nap using a spectrophotometric method are given in table(4).

Table (4): Optimum conditions for the detrmination of Nap using the spectrophotometric method.

No.	Parameter	Value
1	Conc. Of $KMnO_4$	0.01M
2	Conc. Of NaOH	1 M
3	Fixed time	48min
4	Vol . of $KMnO_4$	1.1ml
5	Vol. of NaOH	4.0 ml

Calibration graph

A linear calibration graph for Nap Fig.(6) under the optimized conditions was obtained. Beer's law is obeyed over the concentration range of (0.4-2.8) µg.ml^{-1} with correlation coefficient of 0.9998 and molar absorbance €$_{max}$ 2.3×10^{4} l.mol^{-1}.cm^{-1}.

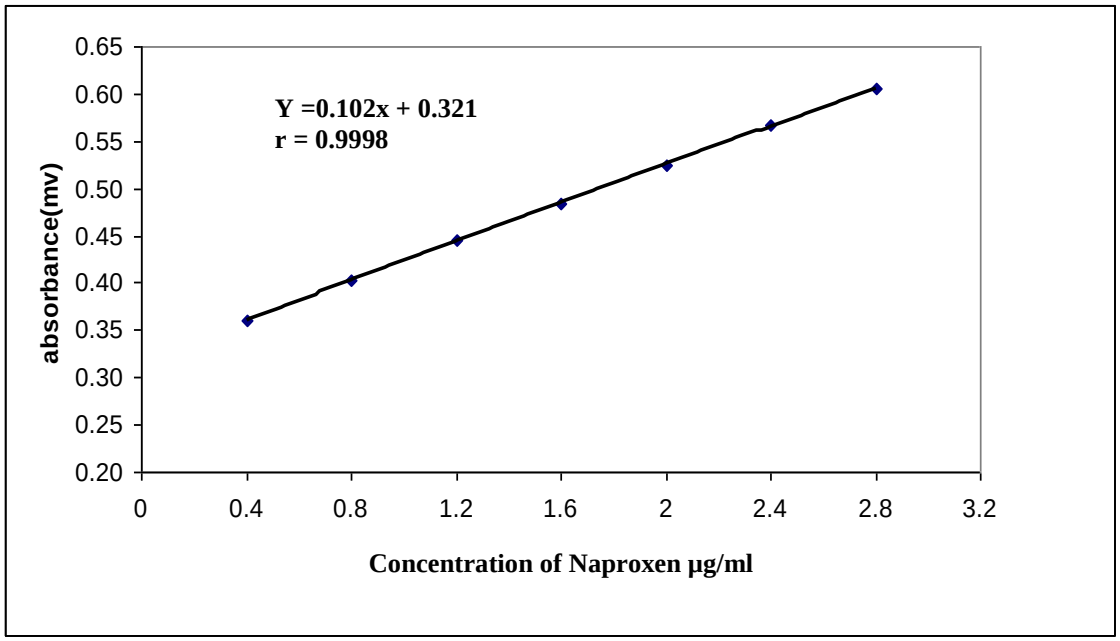


Fig.(6): Calibration graph for the determination of Nap by the spectrophotometric method.

The relative standard deviation percent of the method was 1.32 for 2.8 µg.ml^{-1} based on (6) replicate determinations Table (5) shows the accuracy and precision of the calibration graph. Table (6) shows summary of analytical data for the determination of Naproxen using spectrophotometric method.

Table (5): Accuracy and precision of the calibration graph for the determination of (Nap) by spectrophotometric method.

Added amount µg/ml	Recovery%	Average recovery(%)	RSD%
0.4	99.89	99.43	3.12
1.6	99.54		1.94
2.8	98.81		1.32

*Average of six determinations

Table (6): Analytical data for the determination of Nap by the spectrophotometric method.

Analytical data	Value
Linear range	0.4-2.8 µg. ml^{-1}
Correlation coefficient	0.9998

Regression equation	Y=0.102x+0.321
RSD %	1.32 %
Detection limit	0.281 μg. ml^{-1}
ε_{max}	$2.3 \times 10^{4} L.mol^{-1}.Cm^{-1}$

Pharmaceutical Application

The proposed method was applied for the determination of Naproxen in tablets. Good precision and recovery were obtained. The method was successfully compared with the British Pharmacopoeia standard method. The results obtained are summarized in table(7).

Table (7): Application of the proposed Spectrophotometric method for the determination of Nap in Pharmaceutical Preparations.

Sample	Recovery %		RSD %
	Proposed method *	Standard method	
Pure Naproxen	98.8	99.1	1.32
Naprosyn	98.0	97.3	1.29

*Average of six determinations

Procedure (2) : Determination of Naproxen -HCl using a new FIA- CL method.

General procedure for the new FIA – Cl method .

The FIA-CL scheme is outlined in Figure(1). Various concentrations of Nap were prepared in Co^{2+} (0.5 μg.ml^{-1}) solution. 180μL aliquot of each solution was injected through the sample loop into the stream of Co^{2+} solution, which then was combined with luminol and hydrogen peroxide streams in the flow cell which situated in front of the photomultiplier tube (PMT) .

Results and discussion

The chemiluminescence of luminol–hydrogen peroxide–Co^{+2} system is very intense (Martindule ,1993). However, in this work, trace amounts or Nap were found to be strongly activate chemiluminescence signal of this system.

Effect of reagents concentration

The effect of luminol concentration on the net chemiluminescence intensity was studied. Different concentrations of luminol (1×10^{-3}–1×10^{-4} M) are used to establish the best emission intensity–time profile that can be

obtained. Fig.(7) shows that (5×10^{-4}M) of luminol is the optimal concentration.

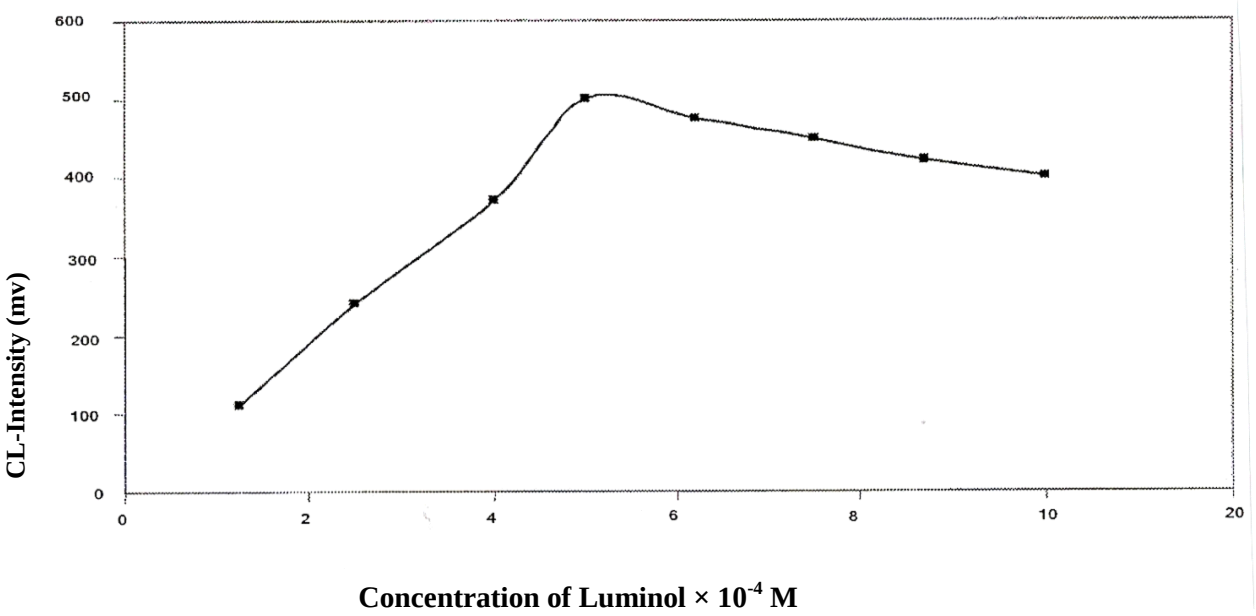


Fig. (7): Effect of concentration of Luminol on the CL-intensity.

The effect of H_2O_2 concentration was investigated; from the results of Fig. (8), the concentration of (1×10^{-2} M) H_2O_2 was selected to be the

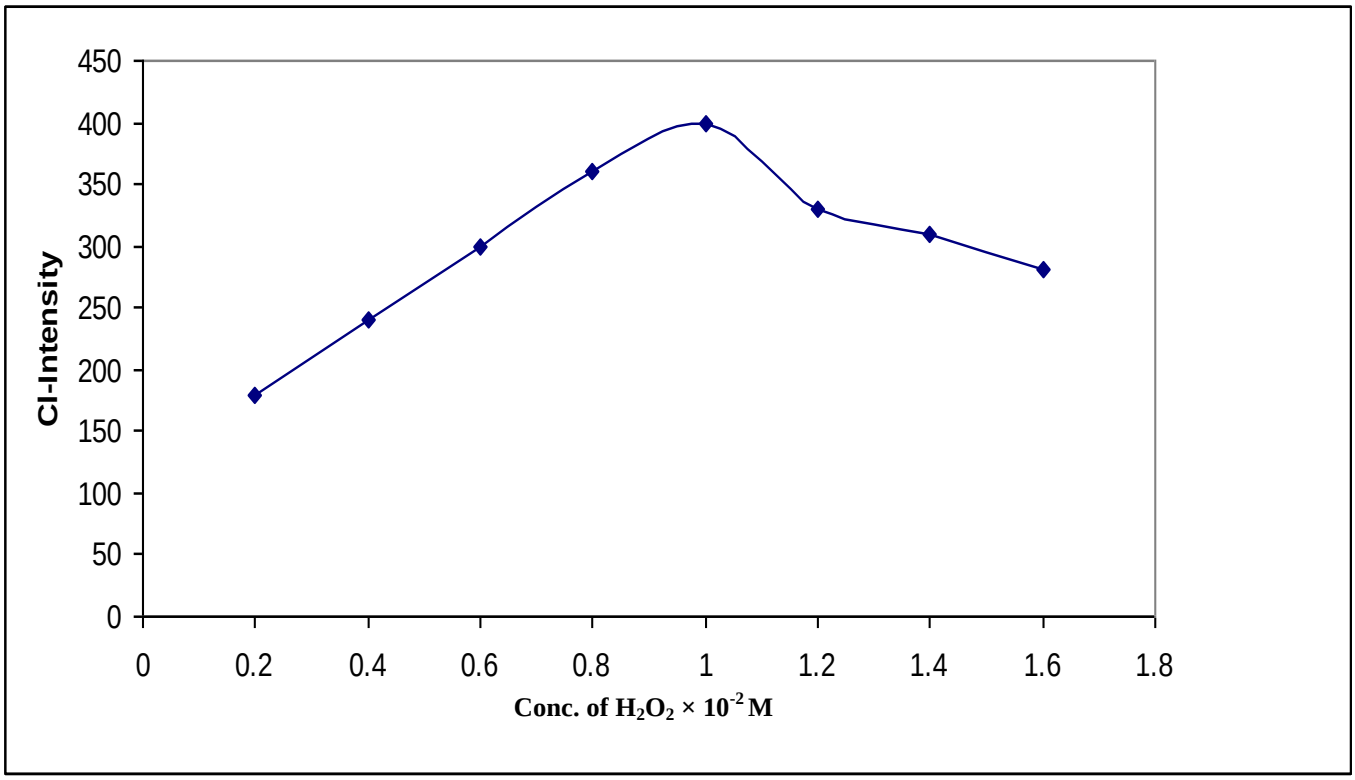


Fig. (8): Effect of concentration of H_2O_2 on the CL-intensity.

Effect of Sulphuric acid and Cobalt Concentrations

The effect of the acidity of cobalt Co^{2+} solution was also studied; three concentration of cobalt Co^{2+} (0.2, 0.5, 0.7) μg. ml^{-1} with varing concentrations of (1×10^{-3}-10×10^{-3}) M of H_2SO_4 were investigated. The best intensity was obtained at the concention of ($5x10^{-3}$)M of H_2SO_4 .

The effect of cobalt concentration was also studied, fig. (9) Shows that the suitable Concentration of Co^{+2} is 0.5 μg / ml^{-1}**.**

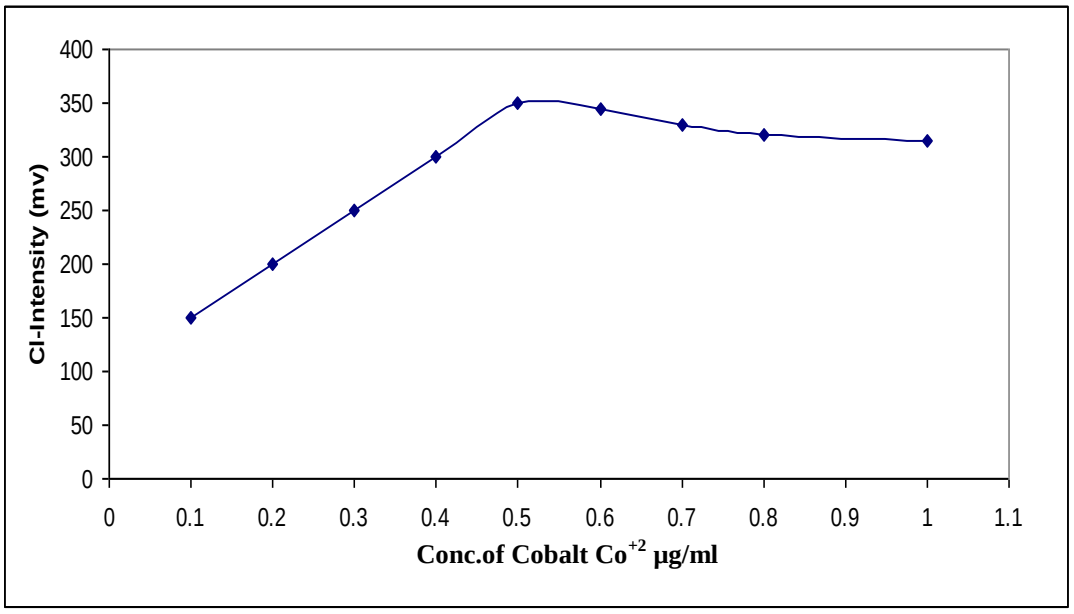


Fig. (9): Effect of Cobalt concentration on the CL-intensity.

It is worth noting here that as the concentration of H_2SO_4 increases the CL intensity increases, this is because of increasing the catalysed oxidation of luminol by H_2O_2 (Martindale, 1993). At $1x10^{-2}$M H_2SO_4 the CL intensity decreases, this fact can be attributed to the cleavage of the formed fluorescent compound. Table (8) shows that as the concentration of H_2SO_4 increases, the time of analysis decreases accompanied with decreasing band width .

H_2SO_4 Concentration (M)	Base band width (mm)	Time of analysis (sec)
$1x10^{-4}$	28.0	78.0

$5x10^{-4}$	25.0	70.0
$1x10^{-3}$	20.0	58.0
$5x10^{-3}$	14.0	50.0
$1x10^{-2}$	9.7	46.0

Table (8): Effect of sulphuric acid concentration on band width and time of analysis .

Effect of flow rate

A flow rate ranged from (1-8) ml/ min was investigated. Table (9) shows that the CL intensity increased with the increase of flow rate. However, a flow rate of 4ml/min is recommended for all streams because of satisfactory CL intensity, less reagent consumption with reasonable analysis time .

Table (9): Effect of flow rate on the emission intensity , analysis time and peak width using 0.5 µg. ml^{-1} of Co^{+2}

Flow rate ml/min	Intensity (mv)	Analysis time (Sec)	Peak width mm
1.0	218	73	24
2.0	288	45	16
3.0	475	35	8
4.0	540	30	6
5.0	660	26	7
6.0	700	24	6
7.0	730	22	5
8.0	800	18	4

Recommended Analytical Conditions

A Summary of the optimum experimental conditions for the determination of Nap in pharmaceutical preparations is on table (10) .

Table.(10): The recommended analytical conditions for the determination of Naproxen using FIA – Chemiluminescence system

Parameter	Recommended value
Conc. Of luminal	$5x10^{-4}$ M
Conc. Of H_2O_2	$1x10^{-2}$ M
Conc. Of Co^{+2}	0.5 µg. ml^{-1}
Conc. Of H_2SO_4	$5x10^{-3}$ M
Flow rate	4 ml/ min
Volume of Co^{+2} injected	180 µl

Calibration graph

A calibration graph of relative chemiluminescence intensity against the Naproxen concentration was established by applying the optimal conditions. The regression equation is (Y=7.9048X-0.131), and the linearity is in the range of (10–45) µg. ml^{-1} of Nap (Fig.10) The analytical data obtained from the calibration graph are summarized in table (11).

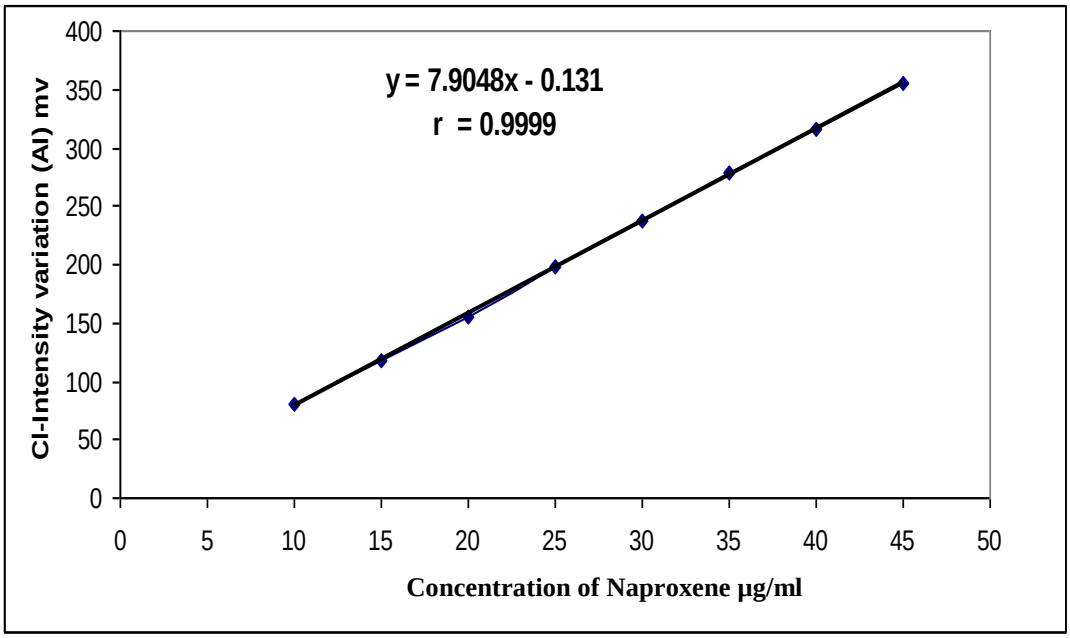


Fig. (10): Calibration graph of relative Chemiluminescence intensity against the Nap concentration.

Table (11): Analytical data for the determination of Naproxen using FIA-CL method.

Analytical data	Value
Linear range	10-45 µg. ml^{-1}
Correlation coefficient	0.9999
Regression equation	Y= 7.9048x-0.131
RSD %	1.12%
Average Recovery	99 %
Detection limit (D.L)	5. 5 µg. ml^{-1}

Interferences

Naproxen is usually formulated in tablets, and injections forms, therefore, the effect of some common excipient substances usually present

in pharmaceutical preparation were investigated. The presence of Croscarmellose Sodium, Magnesium stearate and Iron oxides gave no significant interfering effect on the chemiluminescence intensity of Naproxen .

Application of developed new FIA-CL method for the determination of Nap in pharmaceutical preparations

Naprosyn tablets containing Nap was analyzed using the developed method and the results was compared with the British pharmacopoeia standard method, Table (12).

Table (12): Application of the proposed method for the determination of NaP in Pharmaceutical Preparations .

Sample	Recovery %		RSD %
	Proposed method	Standard method	
Pure Naproxen	99.0	99.5	1.12
Naprosyn	98.4	99.0	1.08

Comparison between the two methods

The two proposed method were compared with other methods as shown in table (13). The value (0.55) of calculated (F test) for FlA-CL/ spectrophotometric methods is much less than the value (4.95) for tabulated (F test) which indicates good agreement.

Table (13): The statistical comparison of results for the spectrophotometric and FIA-CL method

The method	Linearity (µ g/ml)	Correlation Coefficient (r)	Recovery %	RSD%	D.L. µg.ml	Reference
Spectro photometri*c*	0.4-2.8	0.9998	98-99	1.32	0.281	—
FIA-CL	10 - 45	0.999	99	1.12	5.5	—
HPLC	2 – 25	0.9990	98.8 – 102	2.0	0.05	Karidas *et al.* 1993
Liquid chromatography	—	0.9989	98.7	1.8	0.2	Baeyens *et al.* 1991
Synchronous luminescence	—	0.9994	99	2.1	0.002	Hadir, 2008

Spectrometry						
Capillary electrophoresis with UV absorbace	0.5-25	0.9997	98.3	1.6	0.5	Helena *et al.*, 1992
Chemiluminescence	0.1-1	0.9802	99-101	1.5	0.15	Campiglio, 1998
spectrophotometric	4-6mg/dl	—	—	—	12.5mg/dl	Holzbecher,1979

Conclusion

In part (1) a simple kinetic spectrophotometric method was developed for the determination of Naproxen in pharmaceutical preparation by oxidation of the NaP with alkaline $KMnO_4$. The results show good precision and accuracy for the determination of NaP in the range of (0.4-2.8) $\mu g.ml^{-1}$ and correlation coefficient of 0.9998 with detection limit of 0.281 $\mu g.ml^{-1}$, $€_{max}$ 2.3×10^4 L/mol.cm and RSD% of (3.12-1.32)% with 98.0 % recovery.

In part (2) the chemiluminescence was found to be activated by NaP and this is the base of the developed new method. The proposed method offers advantages of simplicity, rapidity, high sensitivity and low reagent consumption. The linearity of this method is (10-45) $\mu g .ml^{-1}$, correlation coefficient of 0.9999 (n=6) and RSD% was 1.65-1.12% with detection limit 5.5$\mu g.ml^{-1}$and recovery (99%) .

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تقدير النابروكسين في المستحضرات الصيدلانية باستخدام المطيافية الضوئية الحركية والحقن الجرياني المنشط بالبريق الكيميائي

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الخلاصة

تضمنت هذه الدراسة على جزئين أساسين، ضم الجزء الأول تطـوير طريقـة طيفيـة لتقـدير دواء النابروكسين تستند على أكسدة هذا الدواء مع برمنكنات البوتاسيوم القاعدية ($KMnO_4$) ومتابعة التفاعل طيفيا" من خلال قياس التغيير بالامتصاص عند طول موجي 608 نانوميتر. وجد أن الزمن اللازم لإكمال التفاعـل هـو 48 دقيقة ومدى الخطية يتراوح بين (0.4 و2.8) مايكروغرام / مل ومعامل الارتبـاط (0.9998)، وحـد الكشـف مقداره (0.281) مايكروغرام / مل أما معامل الامتصاص المولاري 2.3×10^4 لتر/ مول.سم وقـيم الانحـراف القياسي النسبي %RSD تتراوح بين 3.12و1.32%.

أما الطريقة الثانية استندت على تحفيز البريق الكيميائي لنظام (لومينول – كوبلت – بيروكسيد الهيدروجين) بواسطة هذا الدواء وكانت الحدود الخطية للطريقة تتراوح بين (10-45) مايكروغرام / مـل وبمعامـل ارتبـاط (0.9999) وانحراف قياسي نسبي مئوي يتراوح بين 1.65%و1.12%وحد كشف (5.5) مايكروغرام / مل.

طبقت هاتين الطريقتين بنجاح على المستحضرات الصيدلانية وكان الاسترداد المئوي (99-98) % .