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Spectrophotometric Determination of Reduced Nimesulide using 8- Hydroxyquinolinol Reagent in Pharmaceutical Preparations

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ABSTRACT

A simple, sensitive and rapid spectrophotometric method has been developed for the determination of Nimesulide (NIME) in pure as well as in dosage form is described. The proposed method is based on the reduction of the nitro group of drug using a novel and versatile reduction system comprising iron metal and hydrochloric acid. The resulting amine was then diazotization and coupling 8-Hydroxyquinolinol in alkaline medium to form orange colored chromogene exhibiting absorbance maximum at 480 nm. Beer's law was obeyed in the concentration ranges 5 – 250 µg/10 ml, i.e. 0.5-25 µg/ml and the correlation coefficients is 0.9996 with a molar absorptivity of 1.378×10^{4} $l.mol^{-1}.cm^{-1}$ and Sandell's sensitivity index of 0.022 $\mu g.cm^{-2}$, a recovery of 99.36 to 101.64 and relative standard deviation of ±0.40 to ±1.24 %, depending on the concentration level. The method has been applied successfully to the determination of Nimesulide in pharmaceutical preparations.

Keywords: *Nimesulide , reduction , diazotization-coupling , 8-Hydroxy quinolinol , Spectrophotometry.*

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التقدير الطيفي للنميسولايد المختزل باستخدام الكاشف 8- هيدروكسي كوينولينول في المستحضرات الصيدلانية

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الملخص

تم تطوير طريقة طيفية بسيطة وسريعة وحساسة لتقدير النميسولايد بشكله النقي وفي مستحضراته الصيدلانية . تعتمد الطريقة على اختزال مجموعة النايترو في العقار باستخدام منظومة اختزال تتكون من فلز الحديد وحامض الهيدروكلوريك، ثم ازوتة الأمين الناتج بتفاعله مع نتريت الصوديوم ثم اقتران ملح الديازونيوم الناتج مع الكاشف 8- هيدروكسي كوينولينول في محيط قاعدي لتكوين صبغة برتقالية اللون لها اعلى امتصاص عند الطول الموجي 480 نانوميتر . وكان قانون بير خاضعا في مدى التركيز 0.5-25 مايكروغرام/ مل (5-250 مايكروغرام/10 مل) وبمعامل ارتباط 0.9996 والامتصاصية المولارية 1.378×10^4 لتر. مول$^{-1}$.سم$^{-1}$ ودلالة ساندل تساوي 0.022 مايكروغرام.سم$^{-2}$، وباسترجاعية من 99.36 الى 101.64 اما الانحراف القياسي النسبي من 0.40± الى 1.24± اعتمادًا على مستوى التركيز . تم تطبيق الطريقة بنجاح في تقدير النميسولايد في المستحضرات الصيدلانية .

الكلمات الدالة : النميسولايد ، الاختزال ، ازوتة واقتران ، 8- هيدروكسي كوينولينول ، طيف.

1.INTRODUCTION

Nimesulide (NIME) is chemically N-(4-nitro-2-phenoxyphenyl) methane sulfonamide, a well known acidic non-steroidal anti-inflammatory drug (NSAID), analgesic and antipyretic drug and which has highly effective in the treatment of various forms of pain and inflammatory conditions [1-3]. It is a significant and selective COX-2 inhibitor [4,5]. The literature survey revealed that analytical methods reported for the determination of NIME in pharmaceutical preparatios including RP-HPLC [6-10], HPLC-MS [11], HPLC [12-15], GC and

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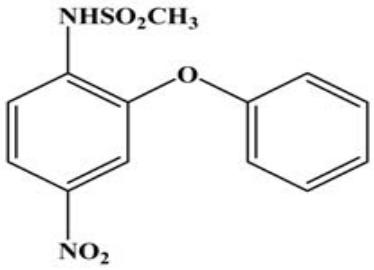
TLC[13], spectrophotometry[16-21], fluorimetry[22], capillary electrophoresis[23], HPTLC[24,25], electro-oxidation[26], ion association titration[27], voltammetry and chronoamperometry[28]. However, there is no publication concerning the analysis of NIME in bulk and liquid dosage formulations by simple UV method. The aim of the present work is to describe a simple, rapid and sensitive UV method for determination of NIME in pharmaceutical preparations .



$C_{13}H_{12}N_2O_5S$, M.wt = 308.3 g.mol^{-1} , m.p = 149 C^o [29,30]

Figure.(1): Chemical structure of nimesulide (4-nitro-2-phenoxymethanesulfonanilide)

2.EXPERIMENTAL

Apparatus

The spectrum and absorbance of the solutions were measured by a Shimadzu UV – Visible recording spectrophotometer (UV – 160) matched with 1.0 cm quartz cells were used for all absorption measurements , Sartorius-BI-2105 balance used for weighting .

Reagents

All chemicals used were of analytical-reagent grade.

Nimesulide solution (500 µg /ml): A 0.0500g of Nimesulide (NDI,Iraq) is dissolved in 25 ml of methanol then it is transferred to a 100 - ml volumetric flask and completed to the mark with the same solvent . The solution is kept in a brown bottle .

Reduced working Nimesulide solution (100 µg /ml): A 20 ml of (500 µg/ml) is taken and followed by addition of 0.3 g of powdered iron and 5 ml of concentrated HCl (1 M) then the solution is filtered, the clear mixture is then transferred to a 100 - ml volumetric flask and is completed to the mark with distilled water .

Hydrochloric acid solution(1M): This solution is prepared by diluting 8.6 ml of the concentrated acid (Thomas Baker)to the mark with distilled water in a 100- ml volumetric flask.

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Sodium nitrite solution(1%): This solution is prepared by dissolving 1g of sodium nitrite (BDH) in distilled water and the volume is completed to the mark in a 100-ml volumetric flask.

Sulphamic acid solution(3%): This solution is prepared by dissolving 3 g of sulphamic acid (Fluka) in distilled water and the volume is completed to the mark in a 100-ml volumetric flask.

8-hydroxyquinoline (HQ) (0.2%): was prepared by dissolving 0.2 g oxine (Indian Drugs and Pharmaceuticals Ltd., Hyderabad, India) in 100 mL of distilled water containing 3 g of sodium hydroxide.

sodium hydroxide (Merck) (2M) was used.

Nimsulide tablets solution (500 µg /ml): Ten tablets were weighed and ground to finely divided powder (each one contains 100 mg NIME), then an accurately weighed amount of powder equivalent to 0.05 g NIME was dissolved in 25 ml of methanol then it is transferred to a 100 - ml volumetric flask and completed to the mark with the same solvent after filtration of the solution. The solution is kept in a brown bottle , then the procedure reduction is followed as above to prepare (100 µg /ml) of reduced Nimesulide tablet solution . A suitable aliquot of solution was taken and the recommended procedure was followed for analysis of the drug.

Reduced nimsulide suppositories solution (500 µg /ml): Weigh and mix the contents of four suppositories (each one contains 100 mg NIME), then an accurately weighed amount of pure NIME equivalent to 0.05 g was dissolved in 10 ml hot distilled water then filtered and washed with 25 ml of methanol then transferred to a 100 - ml volumetric flask and completed to the mark with the same solvent. Procedure for reduction was followed as above. a suitable aliquot of solution was taken and the recommended procedure was followed for analysis of the drug.

3.RESULT AND DISCUSSION

For the subsequent experiments, 100 µg of nimesulide is taken in 10 ml final volumes and absorbance measurements are performed at 480 nm

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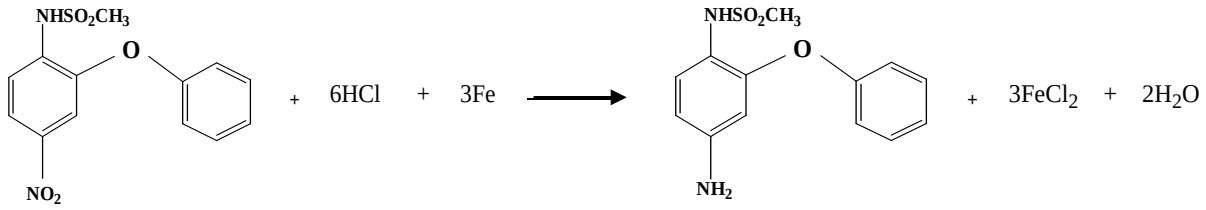
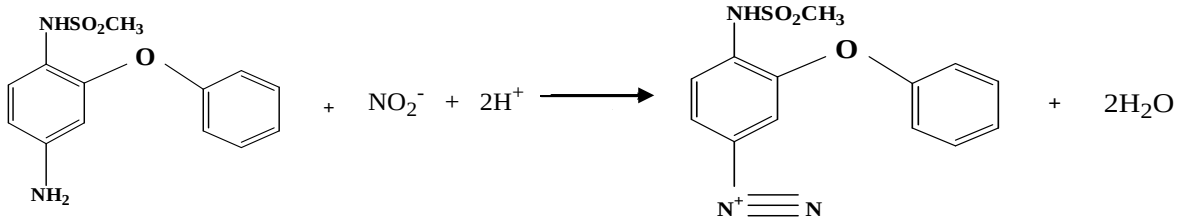
Principle of the method

The method included the following steps: Reduction of nimesulide [31]



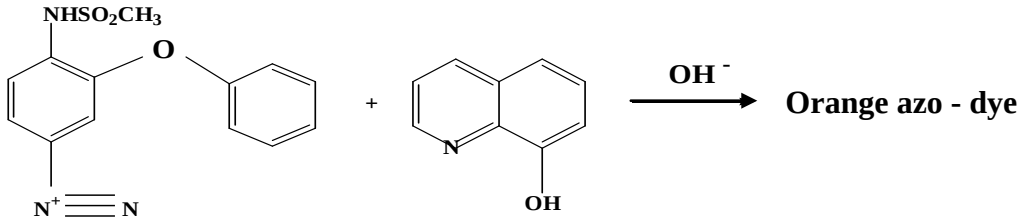
Reduced Nimsulide is reacted with excess nitrite in acidic medium to form the diazonium ion:



The residual nitrite (as nitrous acid) which was undesirable due to its side reaction, such as, nitrosation of coupling agent [32] , was be removed by sulphamic acid:

$$HNO_2 + H_2NSO_3H \longrightarrow N_2\uparrow + H_2SO_4 + H_2O$$

The colored solution is formed by coupling of diazotized NIME with HQ in basic medium.



4.STUDY OF THE OPTIMUM REACTION CONDITIONS

The effect of various parameters on the absorbance of the dye formed was studied , and the reaction conditions were optimized .

Choice of coupling agent

Different coupling agents are used for the reaction with diazotized reduced Nimesulide in basic medium. The results in Table.(1) show that 8-Hydroxy quinolinol gives the more absorbance selected and thus used in all subsequent experimental work.

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Table.(1): Selection of coupling agent.

Coupling agent solution	Absorbance	λ_{max} (nm)	Color of azo dye
8-Hydroxy quinolinol 0.2%	0.643	480	Orange
Resorcinol 0.1%	0.426	490	Orange
α −naphthol 0.1%	0.432	515	Red

Effect of 8-Hydroxyquinolinol amount

The effect of different HQ amount on the colour intensity of the dye has been studied Table.(2).

Table.(2): Effect of HQ amount.

ml of 0.2% 8-Hydroxy quinolinol	Absorbance/µg of reduced nimesulide				
	20	50	100	200	250
1.0	0.153	0.472	0.627	0.790	0.906
1.5	0.138	0.466	0.636	0.751	0.927
2.0	0.173	0.477	0.652	0.793	0.935
3.0	0.162	0.450	0.613	0.753	0.890

From the results, it can be observed that 2 ml of 0.2% HQ solution is the more suitable amount which gives the highest value of formed azo dye absorbance .

Effect of acids on the diazotization

The effect of the amount of different acids (weak and strong) used for the diazotisation of reduced NIME has been investigated. The results indicated that 1.5 ml of 1M HCl gives the highest colour intensity therefore, it has been selected in subsequent experiments. Table.(3).

Table.(3): Effect of acid type and its amount on absorbance of dye.

Acid used (1M)	Absorbance / ml of acid used				
	0.5	1	1.5	2	2.5
HCl	0.557	0.592	0.628	0.612	0.616
H_2SO_4	0.449	0.511	0.543	0.541	0.539
HNO_3	0.453	0.484	0.507	0.496	0.502
H_3PO_4	0.513	0.484	0.469	0.466	0.462
CH_3COOH	0.348	0.374	0.423	0.465	0.418

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Effect of nitrite amount and time

The colored dye reached its maximum intensity when using 1 ml of 1% sodium nitrite solution after 5 minutes as a reaction standing time Table.(4).

Table.(4): The effect of sodium nitrite amount and time on dye absorbance.

ml of 1% $NaNO_2$ solution	Absorbance / minute standing time				
	1	3	5	7	10
0.1	0.452	0.457	0.524	0.588	0.587
0.3	0.536	0.545	0.577	0.541	0.548
0.5	0.564	0.590	0.625	0.621	0.618
1.0	0.624	0.631	0.651	0.650	0.651
1.5	0.540	0.553	0.582	0.562	0.558

Effect of sulphamic acid amount and time

The presence of unreacted nitrite is undesirable in diazotisation reaction.There fore, it should be removed by sulphamic acid which rapidly reacts with nitrite. The results indicated that 0.5 ml of 3% sulphamic acid solution with 2 minutes standing time are considered to be the most suitable Table.(5), and therefore are selected subsequently.

Table.(5): The effect of sulphamic acid amount and time on the dye absorbance.

Amount of 3% sulphamic acid, (ml)solution.	Variable	Absorbance / standing time (min)				
		0	1	2	3	4
0.1	S	0.542	0.513	0.578	0.575	0.572
	B	0.108	0.099	0.091	0.088	0.080
0.3	S	0.639	0.650	0.643	0.655	0.652
	B	0.116	0.102	0.097	0.098	0.072
0.5	S	0.678	0.678	0.688	0.686	0.666
	B	0.032	0.024	0.023	0.024	0.011
1.0	S	0.594	0.619	0.630	0.632	0.616
	B	0.012	0.010	0.016	0.022	0.021

S = Sample , B = Blank

Effect of base type on absorbance of dye

From elementary experiments to this reaction to be clear that the azo-dye was formed just in basic medium therefore the effect of the amount of different bases on the azo-dye's

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absorbance were studied . The results indicated that 1.5 ml of 2M NaOH gives the highest colour intensity therefore, it has been selected in subsequent experiments Table.(6).

Table.(6): Effect of bace type and its amount on absorbance of dye

Base used 2M	Absorbance/ml of Base added				
	0.5	1.0	1.5	2.0	3.0
NaOH	0.601	0.627	0.664	0.647	0.625
KOH	0.589	0.611	0.637	0.652	0.628
Na_2CO_3	0.126	0.405	0.436	0.468	0.568

Effect of time and amount of NIME on absorbance

The effect of time on the development and stability period of the formed colored dye was investigated under optimum experimental conditions described before. The formation of colored dye being complete after mixing the components of the reaction and the absorbance of the colored species remained constant for at 60 minutes Table.(7).

Table.(7): Effect of time and amount of NIME on absorbance

Time (min.)	Absorbance / μg of Reduced Nimsulide per 10 ml		
	50	100	200
5	0.423	0.669	0.819
10	0.436	0.659	0.808
20	0.417	0.655	0.807
30	0.406	0.654	0.805
40	0.402	0.654	0.804
50	0.400	0.653	0.804
60	0.400	0.654	0.804

Final absorption spectra

Absorption spectra of the colored dye formed by coupling of diazotized reduced NIME with HQ reagent in basic medium was recorded against its corresponding reagent blank and show a maximum absorption at 480 nm in contrast to the HQ reagent blank which shows no absorbance in the λ_{max} Figure.(2).

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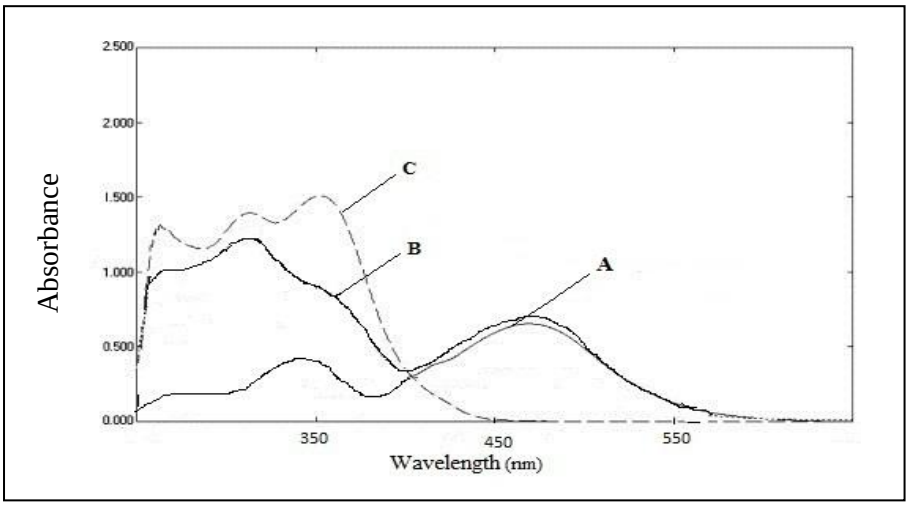

Figure.(2): Absorption spectra of 100 µg Nime treated according to the recommended procedure and measured against (A) blank (B) distilled water (C) blank measured against distilled water .

Procedure and calibration graph

To a series of 10-ml calibrated flasks, an aliquot of aqueous solution containing 5 –250 µg of reduced NIME are transferred, 1.5 ml of 1 M hydrochloric acid is added and the mixture is shaken, then 1 ml of 1% sodium nitrite solution is added and the mixture is allowed to stand for 5 minutes then 0.5 ml of 3% sulphamic acid solution is added with occasional shaking for 2 minutes. A 2 ml of 0.2% HQ solution is added and the volumes are completed to the mark with distilled water, the absorbances are read at 480 nm against blank. The colour was stable for at least 1 hour. The calibration graph is linear over the range 0.5-25 ppm Figure.(3). The apparent molar absorptivity, referred to NIME, has been found to be 1.378×10^{4} $l.mol^{-1}.cm^{-1}$.

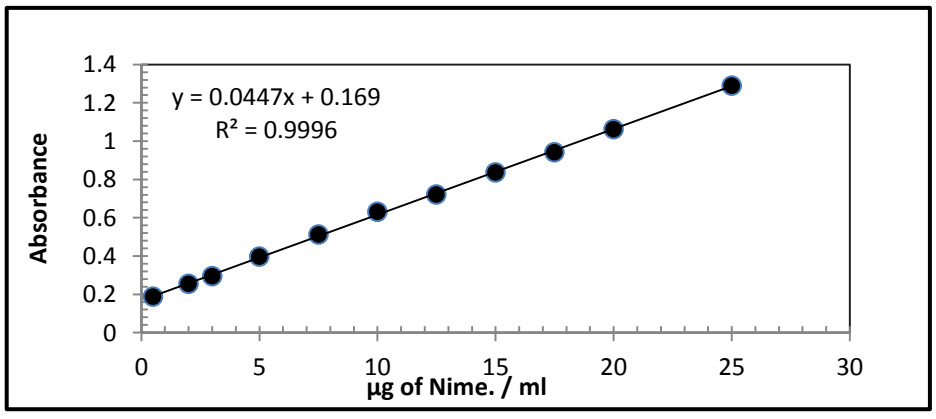

Figure.(3): Calibration graph for NIME determination using HQ as coupling reagent.

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5.ACCURACY AND PRECISION

To check the accuracy and precision of the calibration curve, NIME was determined at three different concentrations (low , medium and high . The results illustrated in Table.(8) indicate that the method is satisfactory.

Table.(8): Accuracy and precision of the calibration curve.

µg NIME / ml	Recovery,%*	RSD%
5	99.49	±0.40
10	101.64	±1.24
20	99.36	±0.50

***Average for five determinations .**

Limit of Detection (LOD) and Limit of Quantification (LOQ)

The LOD determined as the amount of drug was found to be 0.132 µg/ml , LOD is well below the lower limit of the Beer's law range. and the LOQ was determined as the lowest concentration was found to be 0.442 µg/ml in formulation.

$$\text{LOD} = \frac{3\sigma_B}{S} = \frac{3\times1.98\times10^{-3}}{0.0447} = 0.132\ \mu g.ml^{-1}$$

$$\text{LOQ} = \frac{10\sigma_B}{S} = \frac{10\times1.98\times10^{-3}}{0.0447} = 0.442\ \mu g.ml^{-1}$$

S: slope of the calibration curve
σ_B: the ratio of the standard deviation of the blank

Interference

The effect of some foreign compounds, which often accompanied pharmaceutical preparations, was studied by adding different amounts to 100 µg nimsulide in a final volume 10 ml Table.(9).

Table.(9): Effect of excipients on assay of nimesulide .

Interferences	Recovery (%) of 10 µg NIME Per µg foreign compound added		
	200	500	1000
Starch	101.55	102.09	101.89
Glucose	100.91	101.11	100.76
Lactose	99.78	98.39	98.61
Glycine	100.94	101.32	101.94
Fructose	101.36	101.89	102.18

The results in Table 9 indicated that the studied foreign compound do not interfere in the determination of NIME using the proposed method.

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6.APPLICATION OF THE METHOD

The proposed method was successfully applied to determine NIME in its pharmaceutical preparations . The result recorded in Table.(10) , the recoveries were in the range 97.50-101.80 in tablets and 98.68-100.87 in suppositories , that reflected high accuracy, in addition to the high precision indicated by acceptable values of recovery and relative standard deviations .

Table.(10): Analytical applications of the proposed method.

NIME preparation	µg NIME/10 ml	Recovery,%*
Nimsulide tablets 100 mg Ibn Al Haytham pharma. Industries co.-syria	50	98.25
	100	97.50
	200	101.80
Nimsulide suppositories 100 mg Ibn Al Haytham pharma. Industries co.-syria	50	98.68
	100	99.20
	200	100.87

***Average for five determinations .**

7.COMPARISON OF METHOD

Table.(11) shows the comparison between the analytical variable obtained from the present method with those of recent spectrophotometric method.

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Table.(11): Comparison of the method

Analytical parameters	Present method	Literature method[16]	Literature method[33]
Temperature (°C)	At room temperature	At room temperature	At room temperature
λ max(nm)	480	476	400
Medium of method	Basic	Basic	Aqueous
Reducing agent	Iron metal	Zinc metal	Zinc metal
Coupling Reagent	8-Hydroxy quinolinol	Thymol	Phloroglucinol
Beer's law range(ppm)	0.5-30	5-40	4-20
Molar absorptivity (l.mol-1.cm-1)	1.378×10^{4}	--------	7.129×10^{3}
RSD(%)	±0.40 to ±1.24	1.70	1.62
Sandell's sensitivity(µg/cm^2)	0.022	---------	0.001
Color of the dye	Orange	---------	Yellow
Recovery%	99.36 -101.64	97.66 - 102.69	98-101
LOD	0.132	0.99	----------
LOQ	0.442	3.32	----------

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