Spectrophotometric Determination of Diclofenac sodium Using 2,4-dinitrophenylhydrazine in Pure Form and Pharmaceutical Preparations

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Received in:20/April/2015,Accepted in:23/June/2015

Abstract

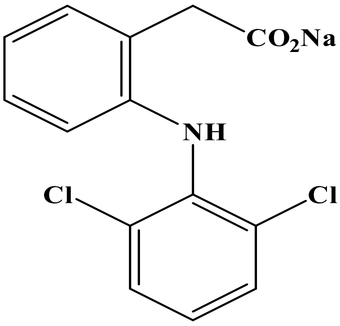
A simple and sensitive spectrophotometric method is described for the determination of diclofenac sodium (DCL), in pure form and pharmaceutical formulations. The method is based on the oxidation of 2,4-dinitrophenylhydrazine (2,4-DNPH) and coupling of the oxidized product with DCL in alkaline medium to give intensively colored chromogen which exhibits maximum absorption (λ_{max}) at 600 nm, and the concentration of DCL was determined spectrophotometrically. The optimum reaction conditions and other analytical parameters were evaluated. In addition to classical univariate optimization, modified simplex method (MSM) has been applied in optimization of the variables affecting the color producing reaction.

Beer's law is obeyed in the concentration range of 0.5-40 $\mu g.mL^{-1}$ with molar absorptivity of 2.0994×10^4 $L.mol^{-1}.cm^{-1}$. The limit of detection was found to be 0.020 $\mu g.mL^{-1}$ and the Sandell's sensitivity value was 0.0152 $\mu g.cm^{-2}$. The proposed method was successfully applied to the determination of DCL in pharmaceutical preparations.

Keywords: Spectrophotometric determination, Diclofenac sodium, 2,4-dinitrophenylhydrazine.

Introduction

Diclofenac sodium or Sodium-2-[(2,6-dichlorophenyl)amino]phenyl] acetate is a non-steroidal anti-inflammatory drug. It is usually found as a sodium or potassium salt as Scheme below. It is used for the treatment of rheumatoid arthritis, ankyllosing spondylitis, osteoarthritis and sport injuries [1].



The mechanism of action is related to the inhibition of the arachidonate metabolites synthesis through cyclooxygenase inhibition. The pharmacological effects of this drug are thought to be related to the inhibition of the conversion of arachidonic acid to prostaglandins, which are the mediators of the inflammatory process. Classical non-steroidal anti-inflammatory drugs (NSAIDs) e.g. indomethacin, diclofenac, flurbiprofen inhibit both COX-1 and COX-2 have been reported to have undesirable gastrointestinal effect mainly attributed to the inhibition of COX-1 [2].

Several types of analytical procedures have been reported for the analysis of diclofenac sodium in pharmaceutical formulation. These procedures include high performance liquid chromatography [3-6], flow injection analysis [7,8], voltammetry [9], potentiometry [10] and spectrophotometric methods [11-16]. Some of these procedures are cumbersome and too costly for routine analysis.

The present study provides sensitivity, precision and accuracy of analysis, thus it offers practical and economical advantages over other techniques.

Experimental

Instruments

- A Shimadzu UV-Vis 1800 Spectrometer (Japan) was used equipped with a quarts cell of 1.0 cm width for the λ_{max} determination and all absorbance measurements.
- Sartorious BL 210 S Scientific balance, Gottingen-Germany.

Materials and Reagents

Pharmaceutical grade diclofenac sodium was received as a powder in pure form as a gift sample from the State Company for Drug Industries and Medical Appliances Samara-Iraq (SDI). All chemicals and reagents used were of analytical grade.

Reagents Solutions

- 2,4-dinitrophenyl hydrazine (2,4-DNPH) [0.08 % (m/v)]: prepared by dissolving 0.08 g of 2,4-DNPH in 2 mL of concentrated sulfuric acid and diluting to 100 mL with distilled water.
- Sodium hydroxide [~10 N]: prepared by dissolving 40 g of NaOH in 100 mL of distilled water.
- Potassium iodate [4 % (m/v)]: prepared by dissolving 4 g of KIO_3 in 100 mL of distilled water.

Standard diclofenac sodium solution (DCL) 1000 μg.mL^{-1}

Standard solution of diclofenac sodium 1000 μg.mL^{-1} was prepared by dissolving 0.1 g and diluting to 100 mL with methanol. Working standard solutions were prepared by appropriate dilution.

Preparation of Solutions for the analysis of DCL in pharmaceutical preparations

i. In Tablet

The content of 10 tablets was accurately weighed individually and grinded into fine powder then mixed well and an average weight was calculated. An amount of the powder equivalent to 0.1054 g of DCL was accurately weighted and dissolved in a minimum volume of methanol and stirred for 10 min for complete dissolution of the drug. The resulted mixture was then transferred into 25 mL volumetric flask and diluted to the mark with methanol to get 1000 μg. mL^{-1}. The solutions were filtered by using filter paper. Working solutions were freshly prepared by appropriate dilution and analyzed by the recommended procedure.

ii. In Ampoule

Each 3 mL of the injection ampoule contains 75 mg of diclofenac sodium. An accurately measured volume 0.4 mL was transferred into a 10 mL volumetric flask and diluted to the mark with methanol to get 1000 μg. mL^{-1} DCL solution. Working solutions were freshly prepared and analyzed by the recommended procedure.

General Standard Procedures

Two different conditions were established by following univariate and multivariate optimization of parameters affecting the formation of the colored product.

According to univariate optimization

Into a series of 10 mL volumetric flasks, 1.5 mL of 0.08 % (m/v) 2,4-DNPH solution and 1.5 mL of 0.15 % (m/v) potassium iodate solution were added to each flask. The resulting diazonium cation was coupled with DCL by adding aliquots of the standard solution 1000 μg. mL^{-1} containing (5-500) μg followed by 0.7 mL of 2 N lithium hydroxide-1-hydrate to each flask and allowed to stand for 10 min. The solutions were then made up to the mark with distilled water. After mixing the solution well, the absorbance of green colored product was measured at 600 nm against the reagent blank.

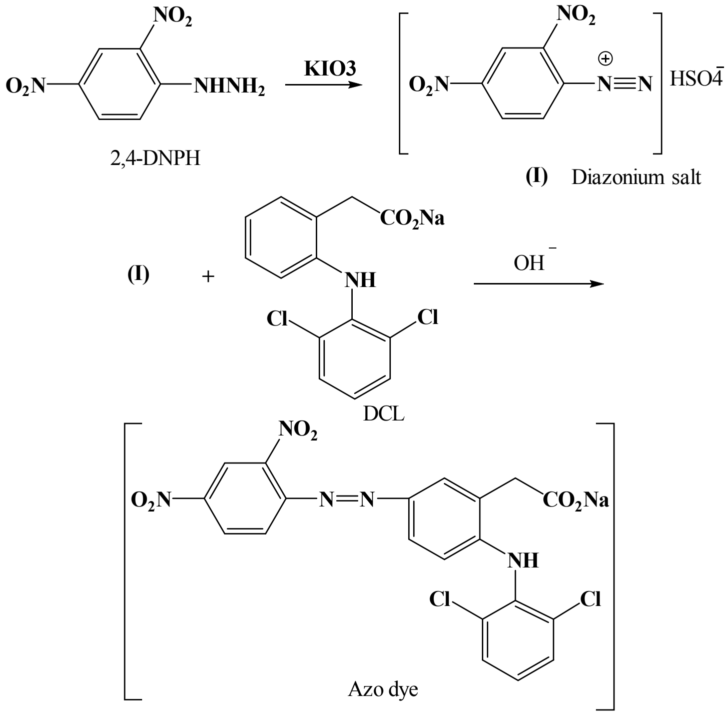
According to Simplex Optimization

Into a series of 10 mL volumetric flasks, 1.5 mL of 0.06 % (m/v) 2,4-DNPH solution and 1.5 mL of 0.4 % (m/v) potassium iodate solution were added to each flask. The resulting oxidized product was coupled with DCL by adding 1 mL of different standard solutions containing (5-400) μg followed by 1 mL of 2 N lithium hydroxide-1-hydrate to each flask. After 10 min, the solutions were making up to the mark with distilled water and mixed well. The absorbance of green colored chromogen was measured at 600 nm against the reagent blank.

Results and Discussion

Absorption spectrum and reaction scheme

The absorption spectrum of the formed colored product that produced from the reaction of oxidized 2,4-DNPH with DCL in alkaline medium under primary test shows a maximum absorption (λ_{max}) at 598 nm. The slightly yellowish color of the blank solution had negligible absorbance at the λ_{max} in which the drug was analyzed. The reaction can be represented as in Scheme below.



Optimization of reaction variables

Univariate method

A systematic study of the effects of various parameters on the development of color products were taken by varying the parameters one at a time and keeping all others fixed. These variables include the concentration of 2,4-DNPH and potassium iodate solutions, the amount and type of base, the coupling reaction time, order of addition, and the stability of the formed resulted product.

Effect of the 2,4-DNPH concentration

The effect of 2,4-DNPH concentrations on the measured absorbance of the formed colored product was done by using 1.5 mL of different concentrations of reagent solution ranged between (0.01 - 0.08) %. The color intensity of the reaction product between the reactants is in a direct proportion with the concentration of 2,4-DNPH, till 0.08 % of the reagent as shown in Figure 1a. Higher concentrations of the reagent solution gave no satisfactory results that is because the product showed a slight turbidity after dilution with distilled water. Therefore, the recommended amount of 2,4-DNPH was chosen to be 1.5 mL of 0.08 % and used for all subsequent measurements.

Effect of Sodium Hydroxide

Different volumes of 10 N sodium hydroxide were studied ranged from (0.2-1.0) mL. The results are presented in Figure 1b, which reveals that the addition of 0.7 mL of 10 N NaOH gave the highest absorbance. Therefore, 0.7 mL of NaOH was used in all subsequent experiments.

Effect of Potassium Iodate

Figure 1c shows a plot of the measured absorbance values against different concentrations (0.1 – 4.0) % of the oxidant in which 1.5 mL of 0.15 % KIO_3 solution yield the maximum absorption. When higher concentrations of the oxidant was used the absorbance starts to decrease sharply, then above 1.5 % the variation of the oxidant concentration shows no significant effect on the absorbance signal. Therefore, 1.5 mL of 0.15 % was used during the subsequent work.

Effect of Coupling Raction Time

Absorbance values were recorded at different intervals ranging from immediate measurement to a waiting period of 30 min. The oxidative coupling reaction was completed in 10 min as shown in Figure 1d.

Effect of Reagents Mixing Order

Three procedures with different orders of component addition were examined. The sequence (1) R + O + D + B gave higher values of absorbance while the sequence (2) R + D + O + B, and (3) D + R + O + B gave an absorbance less by half than in the case of sequence (1) Table 1. This behavior may be explained as follows: the hydrazine group of 2,4-DNPH is oxidized by KIO_3 to diazonium cation that reacts with drug in alkaline medium to give stable derivative of 2,4-DNPH [17-19]. Therefore, sequence (1) was chosen to be applied in the proposed method.

Effect of Type of the Base

Sodium hydroxide, lithium hydroxide, and potassium hydroxide were examined for their effect on the sensitivity and reproducibility of the spectrophotometric results. LiOH was found to be the most useful one to provide the alkaline medium required for this reaction since it gave among the others a reasonable sensitivity with the highest reproducibility, therefore it was used for subsequent work Table 2.

Stability

A slow decrease in the absorbance was observed when the reaction product was left to stand at room temperature for different periods of time. Because of this progressive color reduction, which results in a decrease in the absorbance (≈ 1.4 %, after 3000 s after dilution to final volume), as shown in Figure 2. It is recommended to measure the absorbance immediately or after constant time interval after dilution.

Simplex Method

Multisimplex program was employed to find out the optimum experimental conditions for determination of (DCL). In this method three interest factors (n=3), namely concentration of 2,4-DNPH, KIO_3, and volume of LiOH were chosen as independent variables and the absorbance of the formed azo dye at 600 nm as response was assessed.

The boundary conditions for the three independent variables, delineated above, were set (Table 3) together with their step values. Moreover, four (n+1) arbitrary experimental conditions were chosen, by random selection of values of these parameters within specified boundaries for each (experiments number 1 - 4 in Table 4). The measured absorption signals of these four experiments were feed into the simplex computer program. The program then suggests a new set of conditions to be carried out and the resulted absorbance is fed again to the program and so on. Results in table 4 show the progress of the simplex, which indicates a gradual improvement in the response function. Only 11 experiments were enough to evaluate the proper conditions at maximum response function. The concluded optimum operating conditions for the determination of DCL are 0.06 % 2,4-DNPH, 0.4 % KIO3 and 1 mL LiOH.

Final Absorption Spectra

The absorption spectrum of the colored product was recorded under the optimum conditions (for univariate and simplex method) and showed a maximum absorption at 600 nm against the reagent blank Figure 3.

Calibration Curves and Analytical Data

I- Univariate optimization method

A linear relationship was observed between the absorbance and concentration of DCL ranged from 0.5-50 $\mu g.mL^{-1}$ as shown in Figure 4. The regression equation, correlation coefficient, molar absorptivity, Sandell's sensitivity, limit of detection (LOD) and limit of quantification (LOQ) are calculated and listed in Table 5.

II- Simplex optimization method

Figure 5 and Table 5 show the results for the calibration curve and statistical data. Better optical characteristics for calibration curve and statistical data were obtained under optimum conditions obtained by simplex optimization in comparison with those obtained via univariate method.

Precision and Accuracy

The precision and accuracy of the determination of DCL via the proposed method were studied by calculating the values of percentage of the relative standard deviation (RSD %) and percentage of relative error (Er %), for three replicates at three different concentration levels of DCL drug. The results in Table 6 show acceptable values for accuracy and precision which were obtained.

Interference Studies

To assess the analytical potential of the proposed method, the effect of some common excipients; glucose, lactose, starch and acacia, were examined by carrying out the determination of 10 $\mu g.mL^{-1}$ of DCL in the presence of above compounds. The results are presented in Table 7.

Application in Pharmaceutical Preparation

For verifying the efficiency of the proposed method, it was applied on a real samples with known contents of DCL, tablet (containing 50 mg DCL / tablet) and ampoule (containing 75 mg DCL / 3 mL). The results of the application of the proposed method that are given in Table 8 were satisfactory. The recovery was ranged from (90.35-91.61%) for the analysis of tablet and from (79.72-82.26 %) for the analysis of ampoule.

Conclusions:

Oxidative coupling reaction between 2,4-DNPH after oxidation followed by coupling with DCL in alkaline medium was found to be a simple, sensitive, accurate and economic spectrophotometric method for quantitative determination of (DCL) in pure form and pharmaceutical preparations. The classical univariate and modified simplex method have been used for optimizing the different variables affecting the completion of the reaction. The proposed method offers good linearity and precision.

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Table (1): Variation of absorbance with reactants addition order on the determination of 25 μg.mL^{-1} DCL.

No.	Sequence	Absorbance
1	R + O + D + B	1.645
2	R + D + O + B	0.963
3	D + R + O + B	0.860

Table (2): Effect of different bases on coupling reaction.

Alkaline medium (2 N)	Absorbance	RSD %
LiOH	1.436	3.256
NaOH	0.243	7.761
KOH	Turbid	…….

Table (3): Boundary of simplex of independent variables for determination of DCL.

Variable	Minimum boundary	Maximum boundary	Step size
Conc. of 2,4-DNPH (% m/v)	0.02	0.08	0.01
Conc. of potassium iodate (% m/v)	0.10	0.80	0.05
Volume of lithium hydroxide (mL)	0.50	1.00	0.10

Table (4): Multivariate experiments (simplex) for the determination of 25 μg.mL^{-1} DCL.

Exp. No.	Conc. of 2,4-DNPH (% m/v)	Conc. Of KIO_3 (% m/v)	Volume of LiOH (mL)	Abs.
1	0.08	0.15	0.7	1.436
2	0.04	0.30	0.6	0.533
3	0.02	0.80	0.8	0.600
4	0.06	0.40	0.5	0.090
5	0.06	0.25	0.9	1.261
6	0.06	0.15	1.0	1.141
7	0.07	0.35	1.0	1.421
8	0.08	0.10	1.0	1.394
9	0.06	0.40	1.0	1.573
10	0.07	0.40	1.0	1.379
11	0.06	0.30	0.9	1.213

Table (5): Optical characteristics and statistical data for the determination of DCL by univariate method and simplex method.

Parameter	Value for univariate	Value for simplex
λ_{max} (nm)	600	600
Color	Green	Green
Linearity range (μg.mL^{-1})	0.5-50	0.5-40
Regression equation	**Y=0.059[DCL μg.mL^{-1}]-0.0383**	**Y=0.066[DCL.μg.mL^{-1}]-0.0763**
Calibration sensitivity(mL.μg^{-1})	0.059	0.066
Correlation coefficient (r) %	0.9984	0.9974
Correlation of linearity (r^2) %	0.9968	0.9948
Molar absorptivity (L.mol^{-1}.cm^{-1})	18767.9	20994.6
Sandell's sensitivity (μg.cm^{-2})	0.0169	0.0152
Detection limit (μg.mL^{-1})	0.068	0.020
Quantification limit (μg.mL^{-1})	0.224	0.067

Table (6): Evaluation of accuracy and precision for the determination of DCL by proposed method.

	Conc. of DCL (μg.mL^{-1}) Taken	Found*	Er %	RSD %
For univariate	3	3.070	2.333	2.847
	10	10.074	0.740	3.376
	30	30.232	0.773	3.927
For simplex	3	3.005	0.167	2.250
	10	10.102	1.020	2.549
	20	19.581	-2.095	2.984

*Average of three determinations.

Table (7): Recovery values for 10 μg.mL^{-1} of DCL in the presence of 1000 μg.mL^{-1} of different excipients.

Excipients		Diclofenac sodium Conc.		Recovery (%)
Name	Concentration (μg.mL^{-1})	Taken	Found*	
Glucose	1000	10	9.731	97.31
Lactose			9.925	99.25
Starch			9.686	96.86
Acacia			10.478	104.78

Table (8): Application of the simplex method to the DCL concentration measurements in tablet and ampoule.

Sample	Weight* found (mg)	Concentration (μg.mL^{-1}) Taken	Found*	Recovery (%)	RSD (%)
DCL 50 mg/tablet	45.53	3.000	2.732	91.06	2.542
	45.18	10.000	9.035	90.35	1.977
	45.81	25.000	22.904	91.61	2.230
DCL 75 mg/3 mL Ampoule	61.70	3.000	2.468	82.26	1.147
	60.15	10.000	8.020	80.20	1.500
	59.79	25.000	19.929	79.72	1.498

*Average of three determinations.

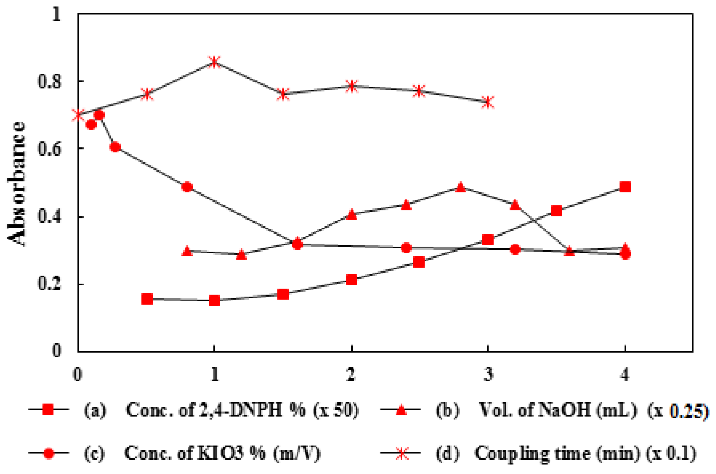

Fig. (1): Effect of (a) 2,4-DNPH concentration, (b) NaOH volume, and (c) KIO_3 concentration (d) coupling time on the color development in the determination of 25 µg.mL^{-1} DCL.

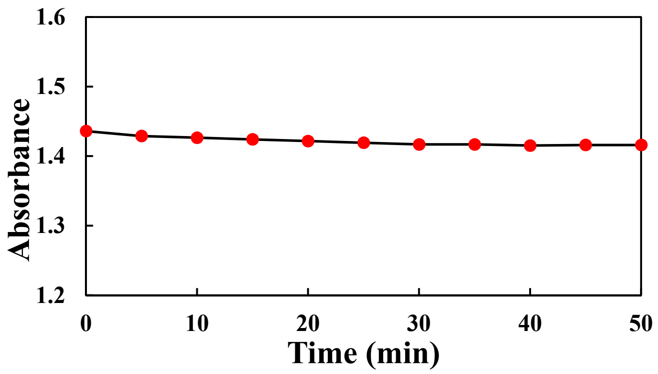

Fig. (2): The stability of colored reaction product in time.

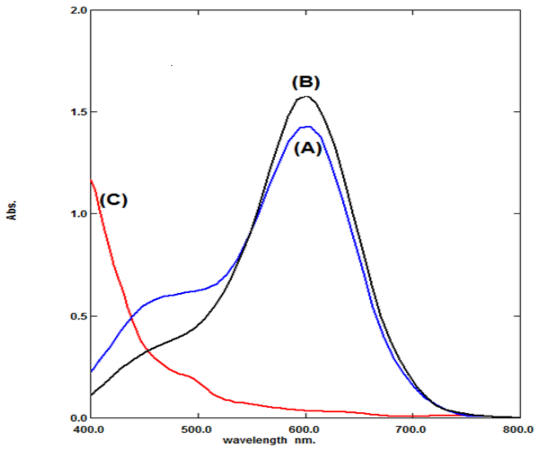

Fig.(3): Absorption spectra of 25 µg.mL^{-1} DCL-2,4-DNPH under optimum conditions, (A) univariate method, (B) simplex method against their reagent blank, and (C) blank solution against solvent.

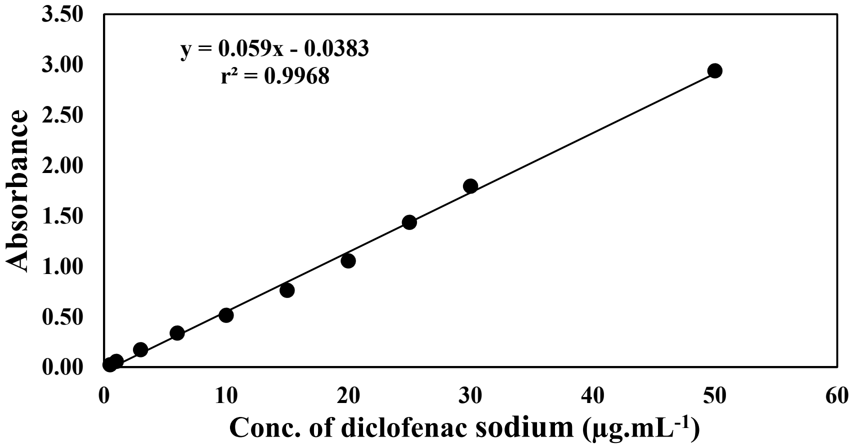

Fig. (4): Calibration curve for the determination of DCL under optimum conditions obtained by univariate optimization.

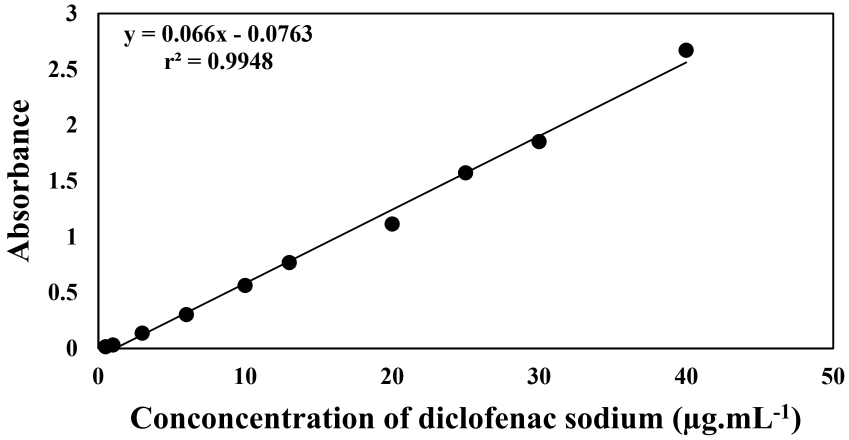

Fig. (5): Calibration curve for the determination of DCL under optimum conditions obtained by simplex optimization.

التقدير الطيفي لديكلوفيناك الصوديوم بأستعمال 2،4-ثنائي نايترو فنيل هيدرازين بشكله النقي وبمستحضراته الصيدلانية

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استلم البحث في 20/نيسان/2015، قبل البث في 23/حزيران/2015

الخلاصة

وصفت طريقة طيفية حساسة وبسيطة لتقدير عقار دايكلوفيناك صوديوم (DCL) بشكله النقي وبمستحضراته الصيدلانية. تعتمد هذه الطريقة على اكسدة 2,4 داي نايترو فنيل هيدرازين ومن ثم اقتران ناتج الاكسدة مع (DCL) في وسط قلوي لتكوين صبغة الأزو ذات اللون الاخضر التي تظهر أعظم امتصاص (λ_{max}) عند 600 نانومتر ومن ثم تم تقدير تركيز الديكلوفيناك صوديوم طيفياً. وقد تم تعيين الظروف الفضلى التي تؤثرفي التفاعل والعوامل التحليلية الأخرى. فضلا عن الطريقة الكلاسيكية بنمط المتغير الواحد طبقت طريقة السمبلكس المحورة لتعيين الظروف الفضلى للمتغيرات التي تؤثر في التفاعل اللوني قيد الدراسة.

تم تطبيق قانون بير على مدى من التراكيز يتراوح بين ($0.5–40\ \mu g.mL^{-1}$) وكانت قيمة معامل الامتصاص المولي مساوية لـ $2.0994\times10^{4}\ L.mol^{-1}.cm^{-1}$ وكان حد الكشف يساوي $0.020\ \mu g.mL^{-1}$ ومعامل ساندل يساوي $0.0152\ \mu g.cm^{-2}$. لقد أمكن تطبيق الطريقة المقترحة بنجاح لتقديردايكلوفيناك صوديوم في المستحضرات الصيدلانية.

الكلمات المفتاحية: التقدير الطيفي، ديكلوفيناك صوديوم، 2،4 -ثنائي نايترو فنيل هيدرازين.