Spectrophotometric determination of Diclofenac Sodium in pure form and in the pharmaceutical preparations

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Abstract
A rapid, simple and sensitive method for the determination of Diclofenac sodium using sodium 1,2-naphthoquine- 4-sulfonate (NQS) as reagent in an alkaline medium (pH 11.9) to form an orange-colored product that was a maximum absorbance at 456 nm. Beer's law is obeyed in the range (8-44 µg/mL), with molar absorptivity (6.965×10^3 L/mol.cm), correlation coefficient 0.9996, and the limit of detection (0.185 µg/mL). The method has been successfully applied to the determination of Diclofenac sodium in pharmaceutical preparations.

Introduction
Diclofenac Sodium, or sodium 2-[(2,6-dichlorophenyl) amino] phenyl acetate, is a broadly used non-steroidal anti-inflammatory drug [1] that is more usually found as sodium or potassium salt [2] for the treatment of inflammatory conditions such as rheumatoid arthritis, osteoarthritis and ankylosing spondylitis [3]. Diclofenac sodium was found to be the most commonly used off-label medicines in UK pediatric surgical wards [4, 5] and exhibit anticancer effects [6].

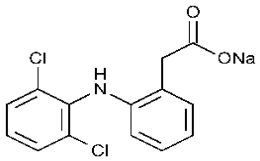

Fig.(1) Chemical Structure of Diclofenac Sodium

Several techniques have been reported for the assay of this drug, whenever pure, in dosage forms, or in body fluids. It has been determined by chromatographic techniques including TLC [7,8], GC [9], HPLC [10,11–16], and reverse-phase liquid chromatography[17], fluorimetric[18-21], colorimetric[22], capillary electrophoresis[23-25], gravimetric[26], diffuses reflectance [27], and spectrophotometric UV [28, 29], spectrophotometric visible [30, 39].

Sodium 1,2-naphthoquinone-4-sulphonate(NQS) has been used as a chromogenic reagent for the spectrophotometric determination of many pharmaceutical amines. It is a popular spectrophotometric reagent due to its efficient reactivity with both primary and secondary amines, and high reaction rate [40-42].

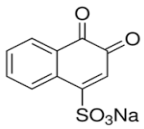

Fig.(2) Chemical Structure of (NQS) reagent

Materials and methods
Apparatus
UV-VIS spectrophotometer single beam from A&E Lab (UK) -S60- Series with 1 cm quartz cells, pH meter from (Senz pH tester, China), Balance from Mettler AB 104-S (Switzerland).

Materials
Diclofenac Sodium from (Awa Medica Company-Erbil-Iraq), Sodium 1,2-naphthoquinone-4-sulphonate (NQS) from BDH, sodium hydroxide (NaOH) from (GCC), Ethanol from (Scharlau).

Solutions
Diclofenac Sodium Stock solution (1000 µg/mL): An accurately (0.1gm) of (DS) standard was dissolved in (100 ml) ethanol.

NQS (1×10^{-2}M): was prepared by dissolving (0.2602 gm) of NQS in (100 ml) water, the solution was freshly prepared and protected from light during storage.

NaOH (1M): was prepared by dissolving (4 gm) of NaOH in (100 ml) distilled water.

Procedure
A 2.0 ml of 500 µg/mL of (DS) was transferred into 25 ml volumetric flask, 4.0 ml of 10^{-2}M (NQS) was added and followed by 1.0 ml of NaOH 1M. After (15 min.), the volume was completed to volume with distilled water, and the resulting solution was measured at 456 nm against reagent blank treated similarly.

Procedure for stoichiometric ratio
The reaction stoichiometry between the studied drug and NQS has been determined spectrophotometrically by applying molar ratio and continuous variation methods. In the former method, equimolar solutions of (DS) and NQS (1×10^{-2} M) were used. Different aliquots of NQS were added to fixed aliquots of drug solution -total volume (25 ml) and the absorbance was measured at 456 nm against the reagent blank treated similarly. While in the latter method, a series of DS-NQS solutions was kept at (5 ml) (0:5, 0.5:4.5, 1:4, 1.5:3.5, 2:3, …… 5:0). The absorbance of the resulting solutions were measured at 456 nm against the reagent blank treated similarly.

Results and discussion
Absorption spectra of DS-NQS system against reagent blank in an alkaline medium at room temperature (25°C) producing an orange colored product which absorbs maximally at 456 nm (Fig. 3), and reagent blank against water (Fig. 4).

ISSN: 1813 – 1662 (Print)
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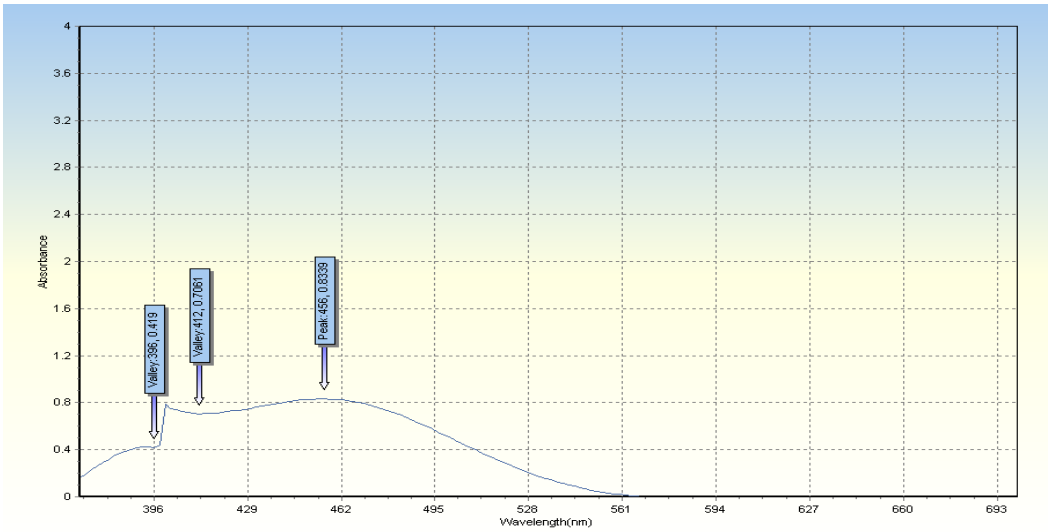

Fig. (3) Absorption spectrum of DS-NQS system against reagent

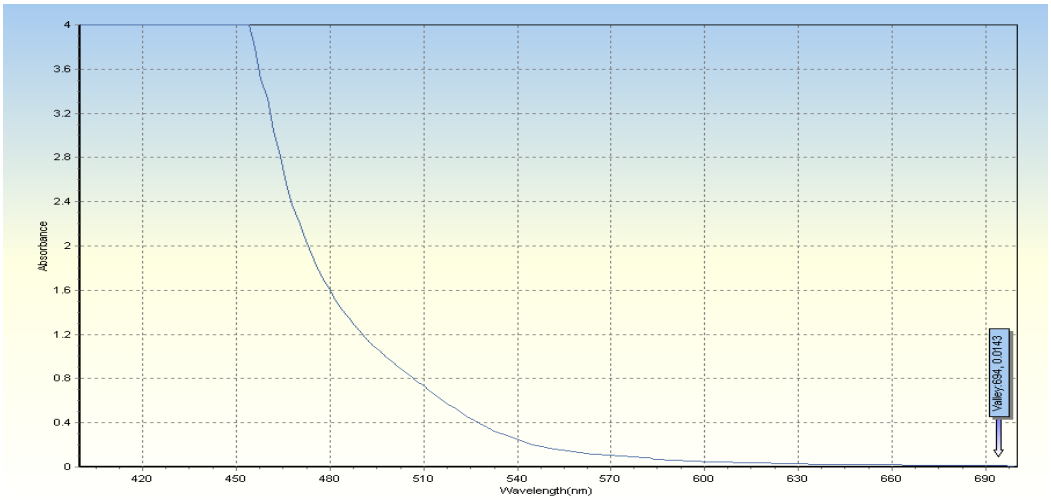

Fig. (4) Absorption spectrum of reagent blank against distilled water

Optimization of reaction variables

In order to establish optimum experimental conditions, necessary for rapid and quantitative formation of colored product with maximum stability and sensitivity, the effect of various parameters such as volumes of NQS, addition of alkaline medium, reaction time and the stability of colored product were studied at room temperature (25^0C).

Calibration curve

The calibration curves for (DS) pure form through complexation with NQS showed excellent linearity at concentration ranges of (8-44 µg/mL).

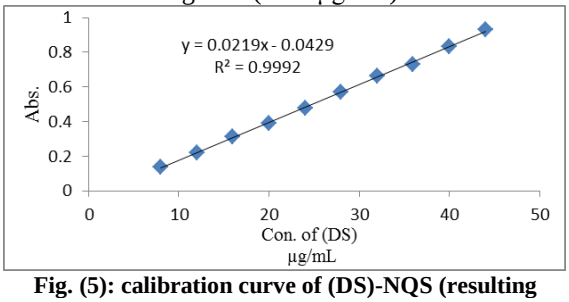

Fig. (5): calibration curve of (DS)-NQS (resulting product)

Effect of NQS concentration

The effect of NQS concentration on the reaction was studied at room temperature (25 ± 5°C). The reaction of (DS) with NQS was dependent on the concentration of NQS reagent. So, the reagent concentration in solution was studied by varying the NQS volume of (1×10^{-2}M) NQS, while the (DS) concentration was maintained constant at 40 µg/mL. The study revealed that the reaction was dependent on concentration of NQS reagent. The absorbance of the reaction solution increased as the NQS concentration increased, the highest absorption intensity was attained when the volume of NQS was 4 ml of (1×10^{-2}M) NQS, and decrease in the absorbance at volume large than 4 ml of NQS (Fig. 6).

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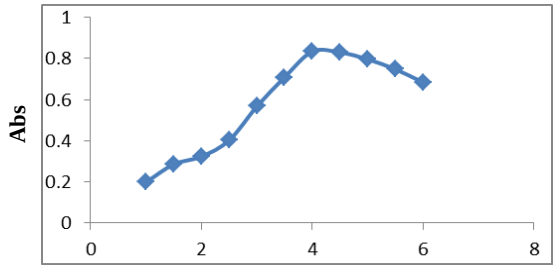

Reagent added (ml)

Fig.(6): Effect of volume of NQS 10^{-2}M in the presence of 1ml of NaOH 1M, the final concentrations were (4×10^{-4}M – 2.4×10^{-3}M)

Effect of temperature

The effect of temperature on the reaction of (DS) with NQS in alkaline medium was studied at different values (20-75°C) by continuous monitoring of the absorbance at 456 nm. It was found that the reaction with NQS was not affected by increasing the temperature (Fig. 7).

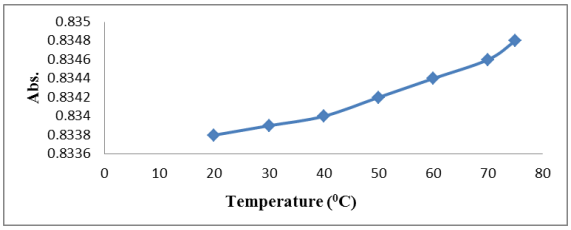

Fig. (7): Effect of temperature

Effect of pH

An alkaline medium was necessary, since the results revealed that (DS) does not react with NQS in acidic media, the results revealed that the absorbances at pH < 8 were close to 0, indicating that under acidity, (DS) has difficulty to react with NQS. Different concentrations from NaOH were tested, Best results were obtained in the case of higher concentrations of NaOH (1M), (Fig. 8).

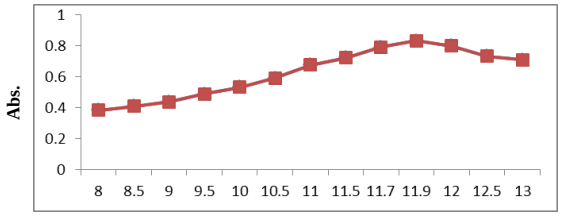

Fig. (8): Effect of pH

Effect of Time

Under the above described optimum conditions, the absorbance-time curve for the reaction of (DS) with NQS in alkaline medium was constructed, and the product remained stable for (2h.) (Fig. 9).

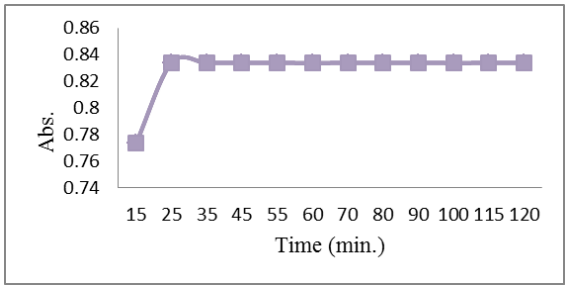

Fig. (9): Absorbance-time curve for the reaction of (DS) with NQS in alkaline medium

Stoichiometry of the reaction

Under the optimum conditions,(cons. of NQS, pH, temperature, time) the stoichiometry of the reaction between (DS) and NQS was investigated by mole–ratio and continuous variation methods [43]. The stoichiometric ratio between NQS and (DS) was found to be 1:1 (Fig. 10,11).

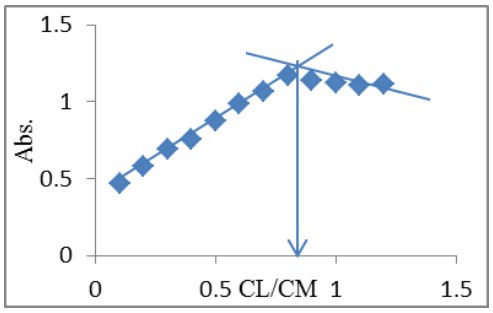

Fig.(10): Mole-ratio method of DS-NQS Complex

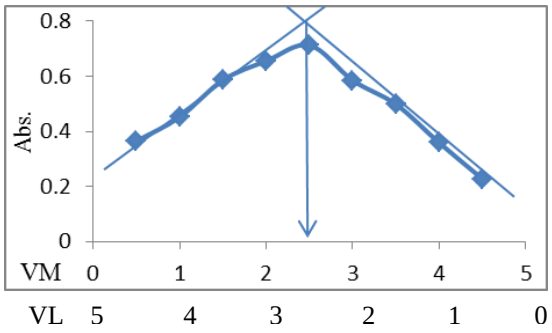

Fig.(11) continuous variation method of DS-NQS complex

Construction of calibration curves

Calibration curves were constructed according to the optimum conditions in Table (1).

Table (1): Optical characteristics of the calibration curve for spectrophotometric determination of (DS) by NQS

Parameter	Value
λmax.(nm)	456
Beer's law (µg/ml)	8-44
Molar absorptivity(l/mol.cm)	6.965×10^{3}
Correlation coefficient (r)	0.9996
Limit of Detection (µg/ml)	0.185
RSD%	0.0136

Application of the method

Twenty tablets were weighed and average weight was calculated. Tablets were crushed into fine powder. An accurately weighed quantity of powder equivalent to 50 mg of (DS) was transferred into a beaker and it was shaken with 50 ml of ethanol and filtered. The filtrate and the washing were collected in a 100 ml volumetric flask. This filtrate and the washing were diluted up to the mark with ethanol to obtain final concentration as 100 µg/mL. The proposed method was successfully applied for the determination of (DS) in various commercial tablets, the results obtained are shown in Table (2).

Table (2): Determination of (DS) in commercial tablets by spectrophotometric method

Formulation	Content (mg) declared	Found(mg) by proposed method	Recovery %
Olfen	50	49.3	98.6
Diclonac	50	49.8	99.6
Naklofen	50	49.2	98.4

Conclusion

The method described here is simple, rapid, convenient and do not require special working conditions unlike many other reported methods. The procedure showed shorter reaction time, stable colored species with inexpensive reagents. The determination can be performed at room temperature and do not require heating step. The proposed method can be applied to assay of (DS) in pharmaceutical preparations.

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التقدير الطيفي للمركب الدوائي (دايكلوفيناك الصوديوم) بشكله النقي وفي مستحضراته الصيدلانية

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الملخص

استخدمت طريقة بسيطة وسريعة وحساسة في تقدير عقار دايكلوفيناك الصوديوم باستخدام كاشف صوديوم 1،2- نفثوكينون-4-سلفونيت في الوسط القاعدي وعند أس هيدروجيني 11.9 لتكوين ناتج برتقالي اللون له اعلى امتصاص عند طول موجي 456 نانوميتر. طبق قانون بير في مدى التراكيز (8-44 مايكرو غرام /مل), وبامتصاصية مولارية (10^3×6.965 لتر/ مول. سم)، ومعامل ارتباط 0.9996، وحد كشف (0.185 مايكرو غرام/ مل). وقد طبقت الطريقة بنجاح في تقدير دايكلوفيناك الصوديوم في المستحضرات الصيدلانية.