J. Flow Injection Anal., Vol. 27, No. 1 (2010) 49–55

Sequential Injection Spectrophotometric Method for the Assay of Anti-inflammatory Diclofenac Sodium in Pharmaceutical Preparations

Salah M. Sultan[1]*, Hatim D. Mohamed[1], Abdulmuttalib Jaber[1], and Nawal A. Alarfaj[2]
[1]Department of chemistry, King Fahd University of Petroleum an Minerals, P.O. Box 2026, Dhahran 31261, Saudi Arabia
[2]Department of chemistry, King Saud University, P.O. Box 22452, Riyadh 11495, Saudi Arabia

Abstract

The method developed in this study utilizes the SIA technique with spectrophotometric detection. Potassium permanganate is used as an oxidant in slightly acidic medium of 6.0 x 10^{-6} mol L^{-1} sulfuric acid and the oxidized form of the drug is monitored spectrophotometrically at a wavelength of 450 nm for quantitative determination. The method has been validated and successfully applied to the assay of the compound formulated in pharmaceutical preparations. The calibration curve was linear within a range of 30 – 135 μg mL^{-1} with correlation coefficients of 0.998 and limits of detection (LOD) and limits of quantification (LOQ) of about 0.24 μg mL^{-1} and 0.7 μg mL^{-1} respectively. The method proved to be of high precision with a relative standard deviation (RSD) of about 1.5 %. Furthermore, the SIA method gave results for real samples (tablets) containing 50 μg mL^{-1} diclofenac sodium in good agreement with those obtained by the standard BP method. No statistical differences at the 95 % confidence level on applying the t-test were observed between the values obtained by the two methods. Thus, the proposed SIA method is reliable, faster than the standard BP potentiometric method and lends itself to automation. The advantages of the SIA technique and the newly adopted method over other conventional techniques and the reported existing standard methods such as rapidity, economy, and automation have been documented. The overall benefit of the SIA technique has also been highlighted.

Keywords Diclofenac sodium, Sequential injection analysis, Drug oxidized form, Potassium permanganate

1. Introduction

Diclofenac sodium, from now abbreviated as (DCS), is chemically known as sodium 2-[(2,6-dichlorophenyl) amino] phenyl] acetate (Fig. 1). It belongs to a class of nonsteroidal anti-inflammatory drugs (NSAIDs). In pharmacologic studies, DCS has shown anti-inflammatory, analgesic, and antipyretic activity. DCS is used in treating osteoarthritis, rheumatoid arthritis, and ankylosing spodylitis. Due to its low solubility [1] it is commercially available as its sodium salt. A number of analytical methods have been developed for the quantitative determination of this drug in dosage forms and in biological samples these methods includes: Spectrophotometry: Rodrigues, J. A. et al. described a spectrophotometric method utilizing the SIA technique based on its oxidation with potassium permanganate in an alkaline medium and the measurement of the green product formed [2]. This method lacks stability of the hydrated species specially the hydroxyl species and requires preparation of the solution the same time of the measurements. Snezana, M. et al. described a kinetic-spectrophotometric method for the determination of micro quantities of DCS. The method is based on a ligand-exchange reaction monitoring the reaction spectrophotometrically by measuring the rate of appearance of the cobalt diclofenac complex at 376 nm [3]. Marcelo M. S. et al proposed a method for the determination of DCS in the presence of B vitamins, based on UV measurements [4]. Mahmoud M. I. et al. developed highly sensitive indirect atomic absorption spectrophotometric (AAS) method for the determination of DCS and some other pharmaceuticals. The method is based on the oxidation of the drugs with iron(III), extracting the excess of iron(III) with diethyl ether and aspirating the aqueous layer containing iron(II) in an air-acetylene flame for quantitative measurements[5]. Agatonović-Kustrin S et al developed a spectrophotometric method in which DCS is determined as a complex with iron(III) . The method is conducted in the presence of ammonium thiocyanate, in the pH range 4.2-6.5, forming a red chloroform extractable (2:1) complex with maximum absorbance at 481 nm [6]. Souza, et al. proposed a spectrophotometric method suitable for pharmaceutical preparations in an aqueous solution of copper(II). The green color complex formed between copper(II) and diclofenac was monitored at 680 nm [7]. A fluorometry method was described by Marcela A. et al for the microdetermination of DCS. The method is based on its reaction with cerium(IV) in an acidic solution and measuring the fluorescence of the Ce(III) ions produced. [8]. Pimenta, A.M. et al. described a method for the determination of DCS utilizing the SIA [9]. Carreira LA et al. developed a method for the determination of DCS in bulk and in pharmaceutical preparations. The method is based on using Eu^{3+} ions as the fluorescent probe. The technique was built around the hypersensitive property of the transitions of the fluorescent probe ion, Eu^{3+}, at 616 nm [10]. Arancibia JA et al. studied the complex formed between alpha-cyclodextrin (CD) and the anti-inflammatory drug DCS in aqueous solution and also in its potential analytical applications. It was corroborated that the fluorescence emission band of diclofenac is significantly intensified in the presence of alpha-CD [11]. Chromatography: Brett J. et al. determined DCS and some other pharmaceuticals in water by Isotope Dilution Liquid Chromatography/Tandem Mass Spectrometry. The method employs solid phase extraction (SPE)

*Corresponding author.
E-mail: smsultan@kfupm.edu.sa

and liquid chromatography/tandem mass spectrometry (LC-MS/MS), using electrospray ionization (ESI) in both positive and negative modes. [12]. Anping Deng et al. described a residue Analysis of the DCS in different water types using enzyme-linked immunosorbent assay (ELISA) and GC−MS. [13]. Potentiometry: The official British pharmacopoeia method is based on the potentiometric titration of DCS versus perchloric acid; in the method 0.250 g of the tablet powder was dissolved in 30 ml of anhydrous acetic acid, and titrating against 0.1 M perchloric acid [14]. Zholt K. et al. described a potentiometric determination of DCS in pharmaceutical formulation by membrane electrode based on ion associate of DCS with the base dye Safranine T and using this ion associate as an electrode active substance for membrane electrode [15]. Electrochemical sensors: Kormosh Z. et al. prepared a novel diclofenac ion-selective electrode [16]. Zholt Kormosh et al. developed a new diclofenac-selective electrode based on an ion associate of diclofenac with a basic dye BIK as a membrane carrier [17]

In the current work, the SIA was utilized for the development of an oxidation-based spectrophotometric method for the assay of Diclofenac Sodium in tablets form. The newly adopted SIA method has the advantages over FIA and other conventional techniques with respect to full-automation, robustness, reagent-saving, accuracy and reproducibility

2. Experimental set up and Procedure

The following part describes the preparation of reagents, acids and drug samples used throughout.

2.1. Reagents and chemicals

Double-distilled de-ionized water was used throughout the preparation of stock and working solutions. All inorganic chemicals used were of analytical reagent grade.

2.1.1 DCS

The analytical grade sample was supplied by SPIMACO Saudi Arabia (in-house reference standard, Lot number 0109188). The sample was previously dried at 50^{O}C in vacuum over magnesium perchlorate.

A stock solution was directly prepared by dissolution in the acidified water. Working solutions were prepared from this stock by further dilutions.

2.1.2 DCS tablets

20 tablets from the proprietary drugs were accurately weighed, crushed and powdered. The amount of the powder containing the appropriate weight to give 150 mg L^{-1} DCS was dissolved in about 40 mL of acidified water; heated for 3 minutes then it was made up to volume in a 100 mL volumetric flask after cooling. Further dilutions could be made from the same.

2.1.3 Potassium permanganate (3.2 x 10^{-3} mol L^{-1})

A standard solution was prepared by dissolving exactly about 0.5 g of dried potassium permanganate (P-279 Lot 746030 Fisher scientific company, USA) in a 1 L acidified water of pH 6.0. The stock solution was standardized with sodium oxalate (S-356 Lot 792406 Fisher scientific company, USA). This solution was used for all the experimental processes.

2.1.4 Sulfuric acid Solution (6.0 x 10^{-6} mol L^{-1})

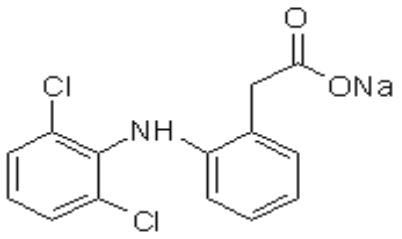

Fig. 1: Diclofenac sodium (DCS) molecular structure

A stock solution of 6.0 x 10^{-6} mol L^{-1} H_2SO_4 (95-98% Specific gravity 1.84 kg L^{-1}, Merck, UK) was prepared the usual way. Working solutions were prepared by dilutions.

2.2. The FIA Apparatus

The second generation FIA instruments of comprising the following parts are used throughout.

The *Alitea USA/FIALab 3000* (Medina, WA USA) has been used in this method. The apparatus consists of a syringe pump, a multi-position valve, a fiber optic spectrophotometer and a PC (Fig. 2).

The syringe pump is a 24,000 steps syringe pump with an optical encoder feedback; 1.5 seconds to 20 minutes per stroke of 5.0 mL size. It is >99% accuracy at full stroke.

The multi-position valve has eight(8) ports with a standard pressure of 250 psi(gas)/600 psi (liquid); zero dead volume; chemically inert; port selection by manual or software control.

The spectrophotometer is the S2000 miniature fiber optic spectrometer pre-set to 200-850 nm wavelength range, UV2 detector, multi-band pass coating, 25 micron entrance slit from Ocean Optics, Inc., USA.

The Z-Flow cell is a 10 mm path length Teflon and Plexiglas or high-grade stainless steel compatible with standard SMA terminated fiber optics.

The Pump tubing of 0.30” ID Teflon type supplied by Upchurch Scientific, Inc. (Oak Harbor, WA, USA) was used for connecting the different units, making the holding coil and the reaction coil 400 and 100 cm long respectively.

2.3. Software Packages

Alitea FIALab software has been used for programming and controlling the SIA system.

Sigma plot, version 1.02 (Jandel Scientific, Erkrath, Germany) was employed for data-handling calculations, multiple regression analysis and constructing graphs.

2.4. Method and Procedure

The procedure steps followed for the assay of DCS in pharmaceutical preparations is described as under utilizing the Alitea FIALab software for controlling different SIA components as shown in Fig 2. Similar programs for preliminary investigations and optimization were designed [18-26].

For the sequential injection process, 5 mL syringe pump (SP) were used to perform both aspiration and dispensing operations.

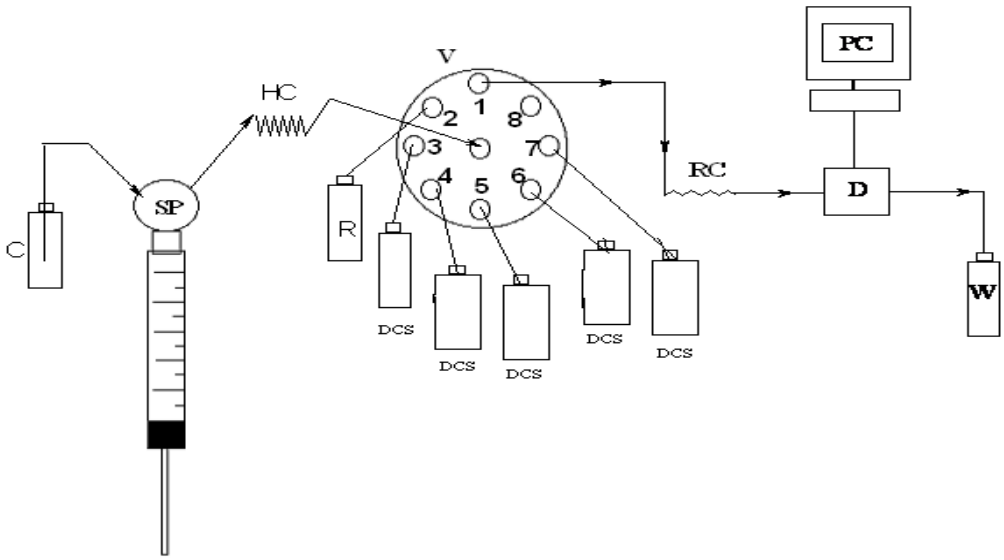

Fig. 2: The SIA manifold comprised of : C. carrier (water); SP. 5 mL syringe pump; HC. holding coil; V. eight ports selector valve; R. $KMnO_4$; DCS. Diclofenac solutions; RC. Reaction coil; D. spectrophotometer; PC. Computer and W. waste.

1- Potassium permanganate (R) was linked to the selector valve through port 2, DCS standard solutions prepared in the range 20 to 250 mg L^{-1} were linked to the selector valve through ports 3, 4, 5, 6, and 7, while the unknown concentration of DCS which is usually the tablet solution was linked to the selector valve through port 8. Tubings were loaded with their respective reagents by an aspiration run.

2- The syringe pump was filled with acidified water as a carrier (C) by directing the two way valve to the (in-position) mode.

3- 500 µL acidified water carrier solution were dispensed to wash the holding coil (HC), Reaction coil (RC), the Z photo cell and to adjust the absorbance of the spectrophotometer to zero.

4- With a 50 µL s^{-1} flow rate, 50 µL potassium permanganate, 50 µL of the drug were sequentially aspirated into the holding coil.

5- A short reverse stroke was performed to allow all reagents to mix with a flow rate of 5 µL s^{-1} followed by continuous dispensing towards the detector (D) for 30 seconds and hence the absorbance (A) was recorded.

6- The steps 2 to 5 were repeated but aspirating the drug sample from the other selector valve ports.

7- The unknown concentration of the DCS solution was directly recorded from the analysis page in the *FIALab* software.

3. Results and Discussion

3.1. Reaction Mechanism

The chemical system is based on the reaction of the permanganate with DCS in a slightly acidic medium of 6.0 x 10^{-6} mol L^{-1} sulfuric acid and monitoring the absorbance peaks produced at 450 nm for the oxidzsed form of the drug utilizing the SIA technique.

The redox reaction between DCS and permanganate resulted in a brown colour absorbing at the maximum wavelength at 450 nm. In the alkaline media or less than 6.0 x 10^{-6} mol L^{-1} sulfuric acid media, the reaction is slower and the DCS species produced are unstable with unacceptable absorbance precision. In higher acidity of more than 6.5 x 10^{-6} mol L^{-1} sulfuric acid media the DCS changes to a white precipitate which is undesirable and blocks the tubing of the SIA system. Therefore, all experiments were conducted in 6.0 x 10^{-6} mol L^{-1} sulfuric acid media. The absorption spectra in Fig. 3, clearly indicates that the absorption maxima of the permanganate appeared as four (4) maxima manifested in plot 2 of the same figure. The four maxima could be attributed to the presence of different oxidation sates of the permanganate in the solution. The UV absorption spectrum of the tetrahedral d^0 complex -MnO_4^{1-} has become a prototype spectrum in transition-metal spectroscopy. A well-resolved experimental spectrum was published in 1967 by Holt and Ballhausen [27]. This prototype spectra is attributed to the vibronic features of the absorption spectrum of permanganate can be reproduced within this vibronic coupling scheme, including the vibrational structure due to Jahn-Teller active normal modes that lead to minima at distorted (lower-symmetry) geometries [28-29].

The spectra illustrated in Fig. 3, also shows that the reaction was time dependent. Plots 3 to 8 clearly indicate that the absorbance maxima of the oxidized form of the DCS increases while the absorbance maxima of the permanganate decreases by time thus resulting in a well defined isosbestic point at point (i).

However, the absorbance of oxidized form of the maxima monitored at the wavelength at 450 nm was found to be reasonable and measurable after a delay time of 30 seconds. It is worth mentioning here that the SIA techniques is unique in measuring the absorbance values at a fixed time with extremely high precision; an advantage which, is incomparable with the traditional spectrophotometry.

Electron spin resonance scan was performed for the reaction products after completion (Fig. 4). The plot is showing very stable sixtet hyperfine splitting indicative of the well known EPR signal feature of manganese(II) [29]. Additionally very weak triplet of sixtet hyperfine splitting were also observed corresponding to the oxidized form of the drug system. Around g = 2.002 another superimposed radical feature peak appeared, presumably due to the oxidized form of the drug as a diradical species. This diradical species was confirmed by performing another EPR scan for the drug with a milder cerium(IV) oxidant

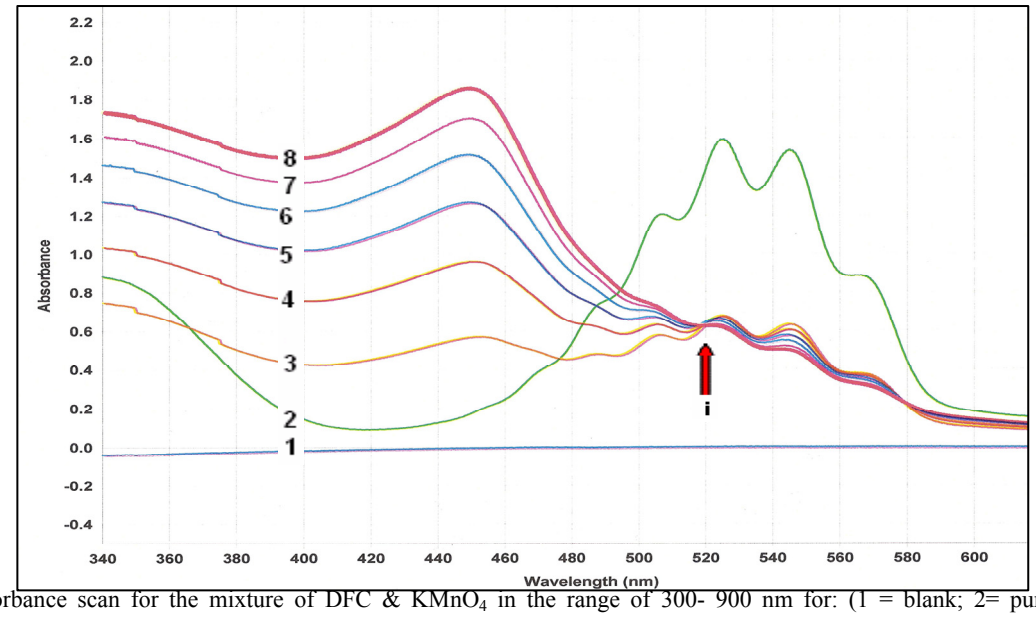

Fig. 3: Absorbance scan for the mixture of DFC & $KMnO_4$ in the range of 300- 900 nm for: (1 = blank; 2= pure potassium permanganate. The spectra were run at different times of: 3 = 20 s ; 4 = 40 s; 5 = 80 s; 6 = 120 s; 7 = 160 s; and 8 = 200 s); i = shows the isosbestic point

in acidic media. This diradical species was found to be too weak to be considered for any quantitative measurements. For this reason, all quantitative measurements and investigations were carried out by monitoring the absorbance of the oxidized species of the drug appearing at 450 nm which is believed to be the quinone imide stable compound in acidic media [12]. The quinone imide compound is believed to be the compound responsible for the generation of the stable sixtet hyperfine splitting of the EPR spectra in Fig. 4. The mechanism of the reaction is proposed and illustrated in Fig. 5 below. The reaction proceeds in two steps, in one step the intermediate dication radical is produced followed by the formation of the stable brown coloured product of the quinone imide in the second irreversible step.

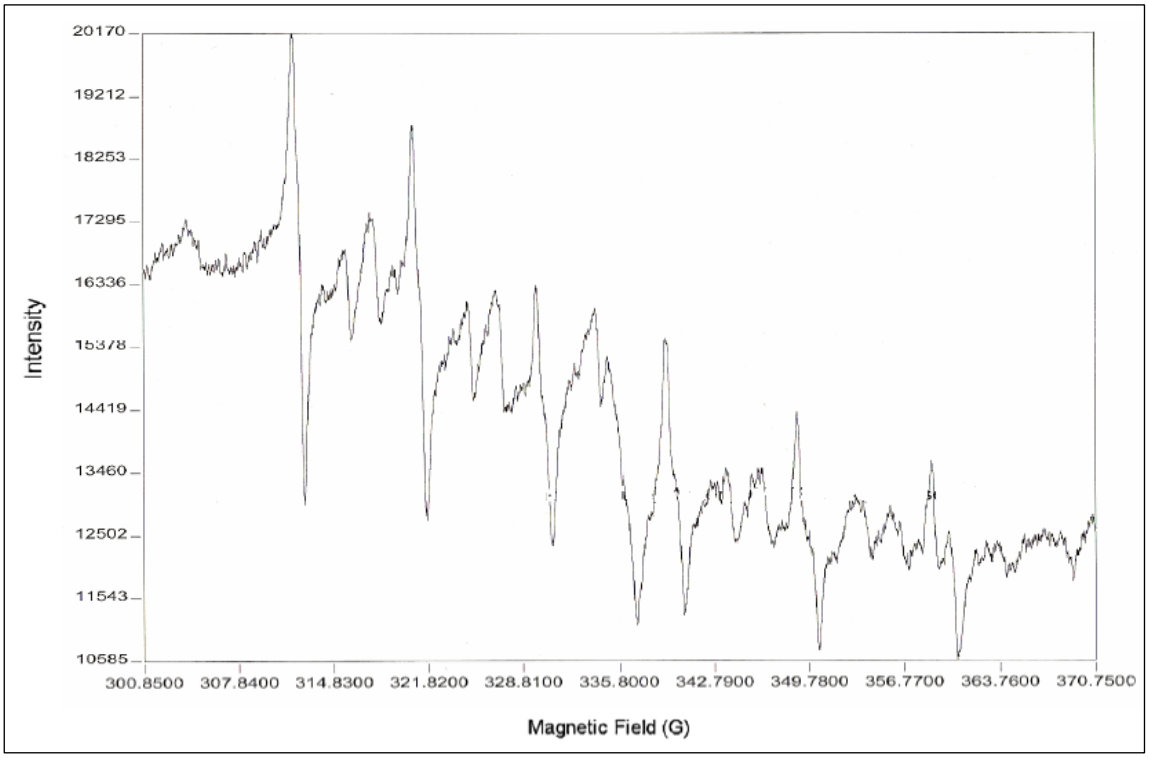

Fig. 4: EPR spectra for the mixture of DCS and potassium permanganate in a slightly acidic media

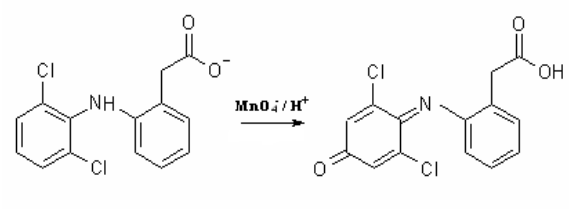

Fig. 5: Proposed reaction mechanism for the oxidation of DCS with potassium permanganate in acidic media

3.2. Optimization

The present SIA method was found to be highly affected by the change in the permanganate concentration and the flow rate of aspiration and dispensing. The simple univariate type of optimization, one-variable-at-a-time, was utilized as manifested in the following paragraphs.

3.2.1 Optimization of the permanganate concentration

A number of experiments were conducted using different concentrations of potassium permanganate (50, 100, 150, 200, 250, 350, 450, and 500 mg L^{-1}), keeping the acid concentration fixed at 6 x 10^{-6} mol L^{-1} and the flow rate constant at 5 µL s^{-1}. The absorbance of the brown colour produced at 450 nm was found to increasing by increasing potassium permanganate concentration (Fig. 6). The optimum potassium concentration was considered to be 250 mg L^{-1} as there was no significant increment in the absorbance values beyond this level. The slight increment in the absorbance is not justifiable enough to sacrifice the damage of the tubing of the whole SIA system.

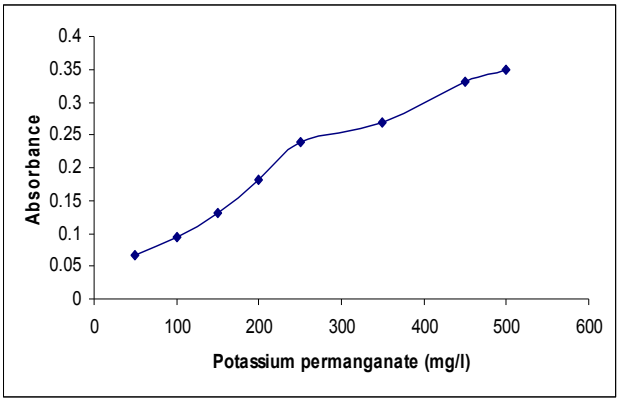

Fig. 6: The effect of $KMnO_4$ concentration on the absorbance peak height of 30mgL^{-1} DFC

3.2.2 Optimization of the Flow Rate

Series of experiments were conducted using different flow rates ranging between 5 and 25 µL s^{-1} keeping the acid and permanganate constant at 6 x 10^{-6} mol L^{-1} and 250 mg L^{-1} respectively. The absorbance of the brown colour produced at 450 nm was found to decrease by increasing the flow rate (Fig. 7). The decrease in the absorbance becomes insignificant when the flow rate becomes more than 15 µL s^{-1}. This observation is in quite agreement with the fact that the reaction is time dependent. The optimum flow rate for the proposed SIA method was considered to be 5 µL s^{-1}.

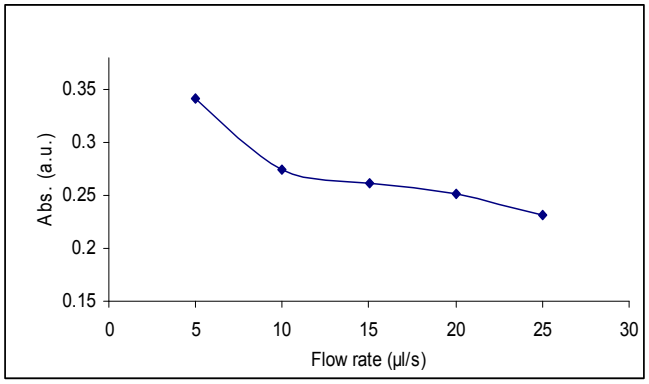

Fig. 7: The effect of the flow rate on the absorbance peak height of 30 mgL^{-1} DCS

3.3 Method Validation

Accuracy, precision, specificity, detection limit, quantitation limit, linearity, and range robustness were the typical analytical performance characteristics considered for the validation of the newly adopted method. All were estimated in the following paragraphs

3.3.1 Accuracy

The same batch of tablet samples containing DCS in dosage forms are quantitatively analyzed for the active drug by both the new SIA method and by the BP standard method [14]. The percentage recovery, standard deviation and finally the t-test values were calculated as in Table 1. The t-test values were always less than the tabulated values indicating high accuracy of the new method. In addition to that the results obtained proved that no interference was encountered from excipients formulating those drugs.

3.3.2 Precision (Repeatability)

Repeatability, or termed intra-assay precision, was evaluated by conducting nine experiments under the same operating conditions over a short interval of time. A mean of 0.362, a standard deviation of 0.004 and a relative standard deviation of 1.1 % were obtained indicating acceptable repeatability.

2.2.3.4 Precision (Intermediate precision)

Intermediate precision was conducted for nine experiments and the results obtained also showed high precision of a mean of 0.355, a standard deviation of 0.005 and a relative standard deviation of 1.0 %.

2.2.3.5 Precision (Reproducibility)

Table 2 shows the results of nine replicates. The mean, standard deviation and relative standard deviation were all calculated and found to be within the accepted criteria of precision indicating good reproducibility.

3.3.3 Linearity and Range

The linearity has been demonstrated for the standard solution over a range of 15 - 150 µg mL^{-1} without adding any placebo. Series of standard solutions of DCS were subjected to the optimized SIA method for the purpose of calibration. Beer's law was found to be obeyed in the concentration range of 30–135 μg mL^{-1} with weighed regression A = 0.0987 + 0.00426 C; the correlation coefficient (r) was 0.998 indicating good linearity.

Table 1 Results obtained by the SIA and British Pharmacopoeia methods for the analysis of DCS in tablet samples

Drug	Supplier	Contents (mg)	Mean recovery ± RSD (%)[a]		t[b]
			SIA method	BP[c] method	
Olfen-50	Mepha Ltd., Aesch-Basel, Switzerland	DCS (50)	97.8 ± 1.3	99 ± 0.15	2.1
Olfen-25	Mepha Ltd., Aesch-Basel, Switzerland	DCS (25)	98.6 ± 1.5	99 ± 0.16	2.2
INFLA-BAN 50	The Arab Pharmaceutical Manufacturing Co. Ltd., Sult- Jordan	DCS (50)	98.3 ± 1.8	99.8 ± 0.14	1.6
INFLA-BAN 25	The Arab Pharmaceutical Manufacturing Co. Ltd., Sult- Jordan	DCS (25)	98.8 ± 1.8	99.9 ± 0.14	1.8
Retard-100	Dar Al Dawa, Na'ur - Jordan	DCS (100)	99.2 ± 2.0	99.7 ± 0.15	2.0
Divido	Tabuk Pharmaceutical Mfg. Co., Tabuk, Saudi Arabia	DCS (75)	98.5 ± 1.6	99.6 ± 0.17	2.1

[a] Relative standard deviation for 5 replicates
[b] Student t-test values (t Critical = 2.9)
[c] British Pharmacopoeia

3.3.4 Limits of detection (LOD) and limits of quantification (LOQ)

The limit of detection (LOD) and limit of quantification (LOQ) were also examined. The LOD and LOQ obtained were 0.24 and 0.7 μg mL^{-1}, respectively, indicating good delectability.

Table 2 DCS reproducibility results for the proposed SIA methods

Sample no.	Concentration (mg/ml)	Absorbance
1	60	0.350
2	60	0.355
3	60	0.347
4	60	0.346
5	60	0.345
6	60	0.337
7	60	0.353
8	60	0.352
9	60	0.345
Mean		:0.348
Standard deviation		: 0.005
Relative standard deviation (RSD)		:1.5 %

Acceptance criteria: Relative standard deviation (RSD): not more than 2 %

4. Conclusion

The experimental results obtained spurred into the development of a new method for the quantitative assay of the anti-inflammatory drug DCS. The method is suitable for the assay of the drug as a raw material and in pharmaceutical preparations such as tablet and suspension formulations. The newly adopted method has the advantage over the official British Pharmacopoeia and other reported standard methods with respect to rapidity, economy, automation using the latest-state-of-the-art Sequential Injection Analysis (SIA) technology.

5. Acknowledgment

Proponents are grateful to the Department of Chemistry, College of Science, King Fahd University of Petroleum and Minerals for funding this research work through SABIC grants under SAB060021. Mr. Hatim D. Mohamed is thankful for allowing him to read for the MS degree by conducting this research project as part of his thesis.

6. References

[1] The Ukrainian State Pharmacopoeia, Kharkiv: Sci. Exp. Pharm. Center, 2004, p 672.
[2] J.A. Rodriguez, G. Islas, M.E. Paez- Hernandez, E. Barrado, J. Flow Injection Anal. 25 (2008) 39-43.
[3] Snezana Mitic´, Gordana Miletic´ , Aleksandra Pavlovic´ , Snezana Tosic´ , and Emilija Pecev, Chem. Pharm. Bull. 55(10) (2007) 1423—1426.
[4] Marcelo M. Sena, Zahra F. Chaudhry, Carol H. Collins, Ronei J. Poppi, Journal of Pharmaceutical and Biomedical Analysis 36 (2004) 743–749.

[5] Mahmoud Mohamed Issa, R'Afat Mahmoud Nejem, Monzer AL-Kholy, Naser Said El-Abadala, Rafik Sobhi and Akila Amin Saleh, J. Serb. Chem. Soc. 73 (5) (2008) 569-576.
[6] Agatonović-Kustrin S, Zivanović L, Zecević M, Radulović D., J Pharm Biomed Anal. 16(1) (1997) 147-53.
[7] Souza, Rafael Leandro de and Tubino, Matthieu, J. Braz. Chem. Soc. 16 (2005) 1068-1073.
[8] Marcela A. Castillo, and Liliana Bruzzone, Analytical Sciences 22 (2006) 431-433.
[9] A.M. Pimenta, A.N. Araújo, M.C.B.S.M. Montenegro, Analytica Chimica Acta 470 (2002) 185–194.
[10] Carreira LA, Rizk M, el-Shabrawy Y, Zakhari NA, Toubar SS., J. Pharm Biomed Anal. 13(11) (1995) 1331-7.
[11] Arancibia JA, Boldrini MA, Escandar GM, Talanta 52 (2000) 261-268.
[12] Brett J. Vanderford, and Shane A. Snyder, Environ. Sci. Technol. 40 (23) (2006) 7312-7320.
[13] Anping Deng, Markus Himmelsbach, Qing-Zhi Zhu, Siegfried Frey, Manfred Sengl, Wolfgang Buchberger, Reinhard Niessner, and Dietmar Knopp, Environ. Sci. Technol. 37 (15) (2003) 3422-3429.
[14] British pharmacopoeia 2007 Volume I & II monographs: Medicinal and pharmaceutical substances, Diclofenac sodium.
[15] Zholt Kormosh, Iryna Hunka, Yaroslav Bazel, Chinese Chemical Letters 18 (2007) 1103–1106.
[16] Z. Kormosh, I. Hunka, Y. Bazel, N. Kormosh A. Laganovsky and I. Mazurenko, J. Iran. Chem. Soc. 4 (2007) 408-413.
[17] Zholt Kormosh, Iryna Hunka and Yaroslav Bazel, Acta Chim. Slov.55 (2008) 261–267.
[18] J. Ruzicka and E. H. Hansen, Flow Injection Analysis - 2nd edition, J. Wiley and Sons (1988).
[19] B. Karlberg and G. E. Pacey, Flow Injection Analysis. A Practical Guide, Elsevier, (1989).
[20] S.M. Sultan, Analyst 117 (1992) 773-776.
[21] S.M. Sultan, Fakhr Eldin O. Suliman, Analyst 121 (1996) 617-621.
[22] Ruzicka, J., and Marshall, G. D., Anal. Chim. Acta 237 (1990) 329-336.
[23] Ruzicka, J. and Gubeli, T., Anal. Chem. 63 (1991)1680.
[24] Guzman, M., Pollema, C., Ruzicka, J. and Christian, G.D.,Talanta,40 (1993) 81-87.
[25] Sultan, S. M., and Nabil Desai Talanta, 45 (1998) 1061-1071.
[26] S. M. Sultan, Y. A. M. Hassan and K. E. E. Ibrahim, Analyst 124 (1999) 917-921.
[27] S.L. Holt and C.J. Ballhausen Theor**.** Chim**.** Acta 7 (1967) 313-320.
[28] J. Neugebauer, E. J. Baerends, and M. Nooijen, J. Phys. Chem. A. 109 (6) (2005) 1168-1179.
[29] M.A. Morsy, M.M. Khaled, Spectrochimica Acta. Part A 58 (2002) 1271–1277.

(Received March 23, 2010)
(Accepted May 21, 2010)