Determination of Diclofenac Sodium in Commercial Pharmaceutical Formulations and Human Control Serum Using a Kinetic-Spectrophotometric Method

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A kinetic method for the determination of micro quantities of diclofenac sodium (DS) is described in this paper. The method is based on a ligand-exchange reaction. The reaction was followed spectrophotometrically by monitoring the rate of appearance of the cobalt diclofenac complex at 376 nm. The optimum operating conditions regarding reagent concentrations and temperature were established. The optimized conditions yielded a theoretical detection limit of 1.29 μg ml^{-1} based on the 3S$_b$ criterion. The interference effects of certain drugs, foreign ions and amino acids upon the reaction rate were studied in order to assess the selectivity of the method. The developed procedure was successfully applied to the rapid determination of diclofenac sodium in commercial pharmaceutical preparations and human control serum. The unique features of this procedure are that determination can be carried out at room temperature and the analysis time is short. The newly developed method is simple, inexpensive, and efficient for use in the analysis of a large number of samples.

Key words kinetic-spectrophotometric method; diclofenac sodium; validation; pharmaceutical preparation

Diclofenac, derived from benzeneacetic acid, is a non-steroidal anti-inflammatory drug (NSAID) that is more usually found as a sodium or potassium salt with potent anti-inflammatory, analgesic, and antipyretic properties. Its mechanism of action is associated principally with the inhibition of prostaglandin synthesis (specifically, inhibition of cyclooxygenase).[1)] Diclofenac is indicated for a variety of conditions such as acute and chronic arthritis, rheumatoid arthritis, osteoarthritis, and ankylosing spondylitis. Diclofenac also has been demonstrated to be an effective migraine treatment in several placebo-controlled studies.[2—4)] It has been determined by a variety of analytical techniques, such as UV/Vis spectrophotometry,[5—11)] fluorimetry,[12—14)] reflectometry,[15)] liquid chromatography,[16—19)] and flow injection analysis with solid-phase spectroscopic detection.[20)] However, as far we know, there is no kinetic-spectrophotometric method for the determination of diclofenac in the literature.

The aim of this work is to develop a simple, rapid, reliable, precise and accurate kinetic method for the determination of diclofenac sodium in pharmaceutical preparations. The method is based on a ligand-exchange reaction. Diclofenac shows complexing ability with cobalt(II). The complex agreed with the empirical formula $CoD_2 \cdot H_2O$ and the cobalt ions are co-ordinated through the carboxylate group of the ligand (diclofenac, D).[21,22)]

Experimental

Apparatus The reaction rate was monitored spectrophotometrically by measuring the rate of change of absorbance at 376 nm. The readings were performed on a Perkin-Elmer Lambda 15 UV/Vis spectrophotometer, connected to a thermo-circulating bath.

pH measurements were carried out using a Hanna Instruments pH meter.

In addition, high precision volume micropipettes (Lab Mate$^+$) of 50, 500 and 1000 μl were used for handling or pipetting the solutions.

The solutions were thermostated at 22±0.1 °C before the beginning of the reaction.

Potentiometric titrations were conducted using a Titrino 716 DMS in dynamic equivalence-point titration (DET) mode. The evaluation of EPs was based on the zero crossing of the second derivate of the titration curve.

Reagents A diclofenac stock solution (1×10^{-3} mol l^{-1} as the sodium salt) was prepared from pharmaceutical 99.9% certified products supplied by a pharmaceutical laboratory (Galenika, a.d., Belgrade, Serbia). The solution was stored at 4 °C. Working solution was prepared daily from this solution, as required, by dilution with water.

Acetic acid solution (HAc, 10 mol l^{-1}) was prepared from glacial HAc (Merck).

1-Nitroso-2-naphthol solution (1×10^{-3} mol l^{-1}) (Merck) was prepared by dissolving a known amount in 5 ml absolute ethanol and diluting it with water (total volume 50 ml).

The stock cobalt(II) solution (1.7×10^{-3} mol l^{-1}) was prepared by dissolving $CoCl_2\times6H_2O$ (Merck) in water.

Ionic strength was kept constant at 0.1 by adding an appropriate amount of NaCl solution (mol l^{-1}).

Analytical grade chemicals and deionised water were used for the preparation of all solutions. All the glassware used was washed with aqueous HCl (1 : 1) and then thoroughly rinsed with running, distilled water, and then finally with deionised water.

Procedure. General Procedure In order to obtain good mechanical and thermal stability, the instruments were run for 10 min before the first measurement. The reaction was carried out in the following way. In a reaction-mixture vessel with four compartments, the solution of 1-nitroso-2-naphthol was placed in one compartment, acetic acid in the second, diclofenac in the third, and cobalt(II), electrolyte for the ionic strength and ethanol (total volume 10 ml) in the fourth compartment.

The vessel was thermostated at 22±0.1 °C and the reaction was initiated by mixing. The reaction solution was put into a cell with a path-length of 10 cm, and the absorbance at 376 nm was measured spectrophotometrically every 30 s over a period of 5—6 min after mixing.

Procedure for Tablets and Ampoules The contents of ten DS tablets (from Galenika; Panfarma) were triturated and homogenized with a pestle. An amount of the resulting powder equivalent to 60 mg diclofenac sodium (DS) was weighed, accurately dissolved in 50.0 ml of water and sonicated for 10 min. This solution was filtered through a 0.45 μm membrane filter (Millipore) and diluted in water to obtain a solution whose expected DS concentration was 60.0 μg ml^{-1}. The contents of ampoules (3 ml per 75 mg, Galenika) were dissolved in 250.0 ml volumetric flasks and diluted with water.

Aliquots of this solution were transferred into vessels covering the concentration range listed in Table 3.

In all cases it was assumed that the actual content of the tablet corresponds to that reported by the manufacturing laboratories.

Serum Sample Preparation Human lyophilised control serum (Lyotrol N) was used. DS was added covering the concentration range listed in Table

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4. The concentration of DS was chosen to match its normal level in human serum.[23,24]

Comparative Method The procedure for a comparative method has been described in the British Pharmacopoeia[25] and U.S. Pharmacopoeia.[26]

Results and Discussion

Mechanism of the Reaction According to Callahan *et al.*,[27] divalent cobalt coordinates with two ligands, liberating two hydrogen ions for each cobalt ion present

$$Co^{2+}+2R(NO)OH \rightarrow Co[RO(NO)O]_2+2H^+$$

Also, it was assumed that the divalent complex is stable in acid medium. Analyses of the Co(II) complex indicated six coordination, actually, Co(II) complex corresponded to $Co[RO(NO)O]_2 \cdot 2H_2O$. The very low oxidation potential of divalent cobalt in acid solution

$$Co^{2+} \rightarrow Co^{3+}+e^- \cdot E^\circ = -1.84$$

would lend some credence to this assumption.

In other cases, diclofenac also showed complexing ability with Co(II).[21,22] The complex agrees with the empiric formula $CoD_2 \cdot H_2O$. The spectroscopy analysis proved the carboxylate group of the ligand is involved in the complexation reaction and provided an octahedral geometry around the metal ion. In the present study, a kinetic method was described based on a ligand-exchange reaction. Its cobalt complex is more stable than that formed with R(NO)OH. The reaction moves to the right and diclofenac was determined by monitoring the rate of appearance of the Co–D complex.

$$Co[RO(NO)O]_2+2D \rightarrow CoD_2 \cdot H_2O+2R(NO)OH$$

Kinetic Studies A tangent method was used to process the kinetic data. The rate of the reaction was obtained by measuring the slope of the linear part of the kinetic curves to the absorbance–time plot (slope=dA/dt).

In order to determine the lowest possible determinable concentration of diclofenac, the conditions needed to be optimised. Therefore, the dependence of the reaction rate on the concentration of each of the reactants was determined.

The effect of the concentration of acetic acid (Fig. 1) was studied in the range 1.0—6.0 mol l^{-1}. It can be seen that the reaction rate was increased with an increasing concentration of acetic acid, reached a maximum, and became constant at 3.0 mol l^{-1}. Above this concentration up to 6.0 mol l^{-1}, the rate of reaction was unchanged. For further work, a concentration of 4.0 mol l^{-1} was selected.

The influence of the concentration of 1-nitroso-2-naphthol is shown in Fig. 2. The reaction rate increased with an increase in the 1-nitroso-2-naphthol concentration from 0.25—2.0×10^{-5} mol l^{-1}. At concentrations higher than 2.0×10^{-5} mol l^{-1}, the reaction rate remained constant. Therefore, a 1-nitroso-2-naphthol concentration of 2.5×10^{-5} mol l^{-1} was chosen as the optimum concentration.

Figure 3 shows a correlation between slope and the Co(II) concentration. It can be seen that the reaction rate increased with an increase in the Co(II) concentration and became constant at 5.1×10^{-5} mol l^{-1}. Thus, a concentration of 6.8×10^{-5} mol l^{-1} in the final solution was used throughout the experiment.

The influence of temperature on the reaction rate was studied in the range 19—31 °C. The reaction rate increased as the

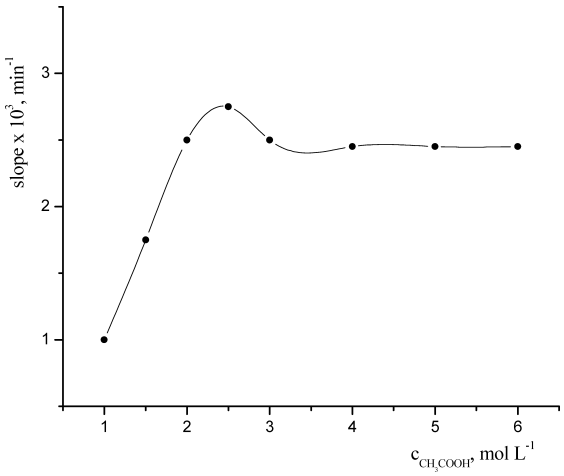

Fig. 1. Dependence of the Reaction Rate on the Acetic Acid Concentration

Initial concentrations: $c_{R(NO)OH}=1.0\times10^{-5}$ mol l^{-1}, $c_{Co^{2+}}=1.7\times10^{-5}$ mol l^{-1}, $c_{DS}=1.0\times10^{-4}$ mol l^{-1}, $t=22\pm0.1$ °C.

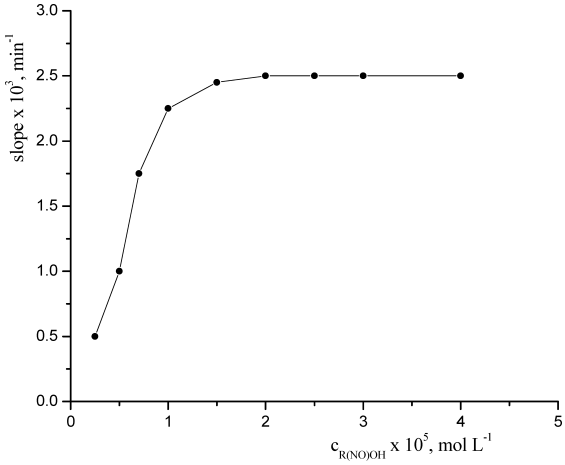

Fig. 2. Dependence of the Reaction Rate on the 1-Nitroso-2-naphthol Concentration

Initial concentrations: $c_{CH_3COOH}=4.0$ mol l^{-1}, $c_{Co^{2+}}=1.7\times10^{-5}$ mol l^{-1}, $c_{DS}=1.0\times10^{-4}$ mol l^{-1}, $t=22\pm0.1$ °C.

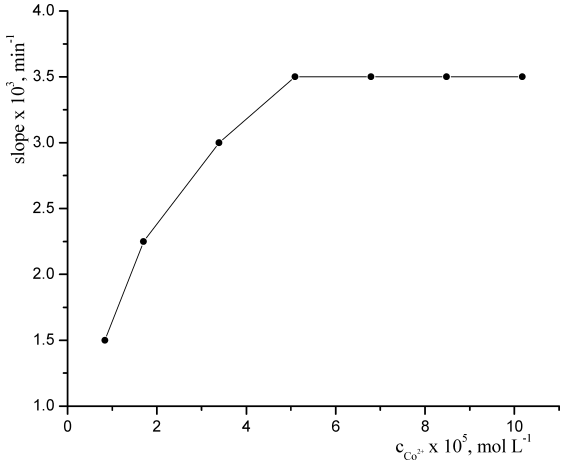

Fig. 3. Dependence of the Reaction Rate on the Cobalt(II) Concentration

Initial Concentrations: $c_{CH_3COOH}=4.0$ mol l^{-1}, $c_{R(NO)OH}=2.5\times10^{-5}$ mol l^{-1}, $c_{DS}=1.0\times10^{-4}$ mol l^{-1}, $t=22\pm0.1$ °C.

temperature increased. It was found that the calibration graph obtained at 22 °C possessed good linearity and it is recommended that the determination be carried out at 22 °C.

The least squares' equation ($y=bx+a$, where b and a are, respectively, its slope and intercept) for the calibration graph and correlation coefficient (r)[28] for the determination of DS in the interval 1.59 to 38.18 μg ml^{-1} under the optimal reaction conditions ($c_{R(NO)OH}=2.5\times10^{-5}$ mol l^{-1}, $c_{CH_3COOH}=4.0$ mol l^{-1}, $c_{Co(II)}=6.8\times10^{-5}$ mol l^{-1}, $t=22\pm0.1$ °C) were calculated:

$$\text{slope}\times10^3=0.08063c_{DS}+0.44301 \quad r=0.9985$$

where c_{DS} is the diclofenac sodium concentration expressed in μg ml^{-1}.

The following kinetic equation for the reaction was deduced on the basis of the graphic correlations obtained.

$$\text{rate}=kc_{DS}$$

k, constant proportional to the rate constant of the reaction.

The equation is valid for the following concentrations: R(NO)OH (2.0—4.0)$\times10^{-5}$ mol l^{-1}, CH_3COOH 3.0—6.0 mol l^{-1}, Co(II) (5.1—10.2)$\times10^{-5}$ mol l^{-1}, DS 1.59—38.18 μg ml^{-1}.

The activation energy for the reaction was calculated from linear regression of the Arrhenius plot ($\log K$ *versus* $1/T$) and found to be 73.31 ± 0.41 kJ mol^{-1}.

The minimum concentration of diclofenac sodium which can be determined by this method was calculated by the method described by Perez-Bendito and Silva[29,30] (3Sbi-criterion).[31] The detection limit was found to be 1.29 μg ml^{-1} and that is 0.8 times less than the real concentration which is 1.59 μg ml^{-1}.

The precision and accuracy of the above system were studied by performing the experiment 5 times for different concentrations of diclofenac sodium. Table 1 shows the results of accuracy and precision of the recommended procedure.

Interference Studies To assess the selectivity of the method, the interference of those species accompanying diclofenac sodium in pharmaceuticals was studied. Table 2 gives the tolerance limits (expressed as w/w ratio), for the species studied on the determination of 19.09 μg ml^{-1} of diclofenac sodium. The maximum level tolerated was taken as that causing a difference in the rate of the activated reaction not larger than 5%. As can be seen, the usual ingredients of powdery drugs (fructose, glucose, lactose) and some of the amino acids (Ser, Phe, Met, Tyr, Trp), will not interfere with the method, because the amounts tolerated are much higher than those usually present in pharamceuticals. It also should be noted that a higher tolerance level exists for the presence of vitamins B_1, B_6 and B_{12}. Ca^{2+}, Mg^{2+}, Zn^{2+}, and salicylic acid interfere only when present in approximately 10-fold excesses. Amino acids (His, Arg, Lys, Ala, Asp, Gly) interfere with the method. More severe interference was observed for Fe^{3+} and Cu^{2+} ions, because they act as catalysts. No interference was found when up to 100-fold concentrations of mannitol, sorbitol, nicotinic acid, or $C_2O_4^{2-}$ anions were present.

Applicability of the Proposed Method The proposed method was applied to the determination of DS in three pharmaceutical formulations using the direct calibration curve. They were treated as described in the Experimental section. As can be seen in Table 3, the results obtained for this method are in accordance with the official potentiometric method. Also, good recovery was observed in the case of serum sample (Table 4), indicating that the constituents of the human control serum do not interfere in any way with the detection of DS. Therefore, the proposed method could be used for the determination of DS in serum samples. The results of the proposed method were statistically compared with those of the official method using a point hypothesis test.[32,33] Tables 3 and 4 show that the calculated F- and t-values at 95% confidence levels are less than the theoretical ones, confirming no significant differences between the performance of the proposed method and the existing method.

Table 1. Accuracy and Precision of the Determination of Diclofenac Sodium

Taken (μg ml^{-1})	Found[a] ($\bar{x}\pm$S.D., μg ml^{-1})	RSD[b] (%)	$(\bar{x}-\mu)/\mu\cdot100$[c]
1.59	1.55±0.08	5.1	−2.5
19.09	18.73±0.67	3.6	−1.9
38.18	38.11±0.34	0.9	−0.2

a) Mean and standard deviation of five determinations and 95% confidence interval, *b*) relative standard deviation, *c*) accuracy of the method.

Table 2. Effect of Foreign Species on the Determination of 19.09 μg ml^{-1} of Diclofenac Sodium

Foreign species	Tolerance level (μg ml^{-1} interfering substance/ μg ml^{-1} diclofenac sodium)
Li^+, K^+	10^3
Fructose, glucose, lactose, Ser, Phe, Met, Tyr, Trp, B_1, B_6, B_{12}, mannitol, sorbitol, nicotinic acid, $C_2O_4^{2-}$	10^2
Ca^{2+}, Mg^{2+}, Zn^{2+}	10
His, Arg, Lys, Ala, Asp, Gly	1
Fe^{3+}, Cu^{2+}	Interference

Table 3. Determination of Diclofenac Sodium by the Kinetic and Existing Method (Potentiometric Titration)

Pharmaceutical preparation	Taken (μg ml^{-1})	DS found by the proposed method[a] ($\bar{x}\pm$S.D., μg ml^{-1})	RSD[a] (%)	Recovery[a] (%)	Existing method[a] ($\bar{x}\pm$S.D., μg ml^{-1})	F-value[b]	t-value[b]
Diklofen retard[c]	20.68	20.55±0.44	2.13	99.37	20.90±0.49	1.24	0.81
Diklofen[d]	25.45	25.36±0.49	1.94	99.64	25.08±0.35	1.96	0.68
Diklofen retard[e]	30.22	30.40±0.5	1.65	100.53	30.33±0.76	2.31	0.1

a) Data are based on the average obtained from five determinations. *b*) Theoretical F-value ($\nu_1=4$, $\nu_2=4$) and t-value ($\nu=8$) at 95% confidence level are 6.39 and 2.306, respectively. *c*) Tablets (from Galenika, a d., Belgrade, Serbia) containing diclofenac sodium 100 mg and excip. *d*) Ampoules (from Galenika a d., Belgrade, Serbia) containing diclofenac sodium 75 mg (3 ml per 75 mg). *e*) Tablets (from Panfarma, Belgrade, Serbia) containing diclofenac sodium 100 mg and excip.

Table 4. Determination of Diclofenac Sodium in Human Control Serum by Standard Addition Method

Proposed method $\mu g\,ml^{-1}$		RSD[a] (%)	Recovery[a] (%)	Official method[a] ($\bar{x}\pm$S.D., $\mu g\,ml^{-1}$)	F-value[b]	t-value[b]
Added	Found[a] ($\bar{x}\pm$S.D.)					
2.0	1.96±0.07	3.77	98.0	1.89±0.06	1.35	1.7
3.18	3.2±0.1	3.19	100.63	3.14±0.08	1.56	1.04

a) Data are based on the average obtained from five determinations. *b*) Theoretical F-value (ν_1=4, ν_2=4) and t-value (ν=8) at 95% confidence level are 6.39 and 2.306, respectively.

Conclusion

In conclusion, the proposed kinetic-spectrophotometric method for the determination of diclofenac sodium in pharmaceutical samples reported in this paper is simple, rapid, and inexpensive, and thus is very appropriate for routine analytical control of pharmaceuticals. Statistical comparison of the results with the official method showed good agreement and indicates no significant difference in accuracy and precision.

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