

Spectrophotometric study of diclofenac-Fe(III) complex

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Abstract

A multifactor optimisation technique is successfully applied to develop a new spectrophotometric method in which diclofenac sodium is analysed and determined as its Fe(III) complex. The effect of simultaneously varying the pH, ionic strength and concentration of colour reagents in the reaction mixture were studied. A four-variable two-level factorial design was used to investigate the significance of each variable and interactions between them. A response surface design was used to optimise complex formation and extraction. It was established that diclofenac reacts with Fe(III) chloride, 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. By applying the methods of Sommer and Job involving non-equimolar solutions the conditional stability constant of the complex, at the optimum pH of 6.0 and an ionic strength $\mu = 0.19\text{M}$, was found to be $10^{6.4}$. Good agreement with Beer's law was found for diclofenac concentrations up to mmol l^{-1} . The nominal percent recovery of diclofenac was 98.8% ($n = 10$). The lower limit of sensitivity of the method was found to be $14.7 \mu\text{g ml}^{-1}$. © 1997 Elsevier Science B.V.

Keywords: Experimental design; Diclofenac sodium; Fe(III) chloride; Ammonium thiocyanate; Complexometry; Spectrophotometry

1. Introduction

Diclofenac sodium (Voltaren) 2-(2,6-dichloro-anilino) phenylacetate has been shown to be an effective and well tolerated nonsteroidal anti-inflammatory agent for the treatment of rheumatoid arthritis. It has been determined by a variety of analytical techniques, the most common being colorimetry [1–3], UV spectrophotometry [4], gas [5–7] and liquid chromatography [8–11]. Other methods include thin-layer chromatography [12,13] and NMR [14].

The gas chromatographic methods for the analysis of diclofenac are based on the formation of an indolinone, a methyl ester, or an acetylated derivative. These compounds are then detected by electron capture detection. HPLC methods have been developed with a single-step extraction and direct UV detection without derivatisation. As spectrophotometric assays offer significant economic advantages over gas and liquid chromatography the aim of the present investigation was to develop a new, sensitive and accurate extractive spectrophotometric method for the determination of diclofenac in its dosage forms.

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Colorogenic reaction with Fe(III) chloride in the presence of ammonium thiocyanate was used to modify the spectrum of diclofenac so that it could be detected in the visible region, well separated from other interfering components in the UV spectrum. The assay was developed using two mathematical statistical models: factorial design and response surface mapping [15]. These methods were used to assess the quality of the complexation between diclofenac and Fe(III) ion quantitatively. Screening experiments were run in order to determine the variables to be investigated and a two-level factorial design was used to evaluate the main effects as well as the interactions between the factors. The results for pH of reaction mixture, ionic strength and concentration of colour reagents and their interactions are discussed below. It is suggested that an experimental design can be used to achieve optimum conditions by simultaneously optimising the values of the variables using response surface mapping. The best set of conditions can then be selected for further validation of the method.

2. Experimental

2.1. Reagents

Diclofenac sodium bulk drug and Toryxil® tablets (100 mg) were obtained courtesy of Dr Baer, KG (Munich, Germany). Fe(III) chloride, ammonium thiocyanate, potassium chloride, acetic acid and sodium hydroxide were obtained from Merck (Darmstadt, Germany).

2.2. Solutions

For analytical purposes a freshly prepared (2.5 mg ml⁻¹, 7.85 × 10⁻³ M) aqueous solution of diclofenac bulk drug was prepared. A sample solution containing 2.5 mg ml⁻¹ diclofenac was prepared by extracting diclofenac from tablets with water. Fe(III) chloride solution (81 mg ml⁻¹; 3 × 10⁻¹ M) was prepared by dissolving 4.055 g Fe(III) chloride in 40 ml water with addition of 1 ml 1 M HCl, followed by diluting the solution up to 50 ml with water. Ammonium thiocyanate

solution (183 mg ml⁻¹, 2.4 M) was prepared by dissolving 9.13 g ammonium thiocyanate in 50 ml water. The ionic strength of the final solution was kept constant at 0.2 M by addition of 2 M potassium chloride solution. Acetate buffer solutions covering the pH range 3.6–6.5 were made by mixing appropriate volumes of 1 M sodium hydroxide and 1 M acetic acid solutions.

2.3. Apparatus

The absorption of each solution was recorded on a Specord M 40 Carl Zeiss Jena Spectrophotometer provided with matched 10 mm quartz cells. Measurements of pH were carried out on a 'Radiometer 22' pH-meter. The pH values were determined with a saturated calomel–glass electrode system.

2.4. Procedure

To 2 ml diclofenac sodium solution placed in an Erlenmeyer flask fitted with a ground glass stopper, 2 ml Fe(III) chloride, 2 ml ammonium thiocyanate, 1 ml 2 M potassium chloride and 3 ml acetate buffer was added. Finally, 5 ml chloroform was added. The Erlenmeyer is stoppered and after shaking the reaction mixture for 10 min, the orange-coloured chloroformic layer is separated in a separation funnel and the absorbance measured at 481 nm against a reagent blank. This procedure was employed for measuring the absorption spectrum and for determination of diclofenac in bulk drug and tablets.

A calibration curve was prepared with 10 standard solutions over the concentration range 0.5–5.0 mg ml⁻¹ (1.57–15.7 mmol l⁻¹). For each solution three experiments were run following the procedure described.

3. Results and discussion

3.1. Absorption spectrum of diclofenac–Fe(III) complex

The absorption spectrum of the extracted complex was measured over 400–600 nm. The com-

plex shows maximum absorbance at 481 nm (Fig. 1) which can therefore be used for analytical purposes. Under the same conditions the chloroformic extract of Fe(III) chloride, ammonium thiocyanate, potassium chloride and acetate buffer solution does not show significant absorption in this spectral region. The maximal production and extraction of complex was attained after 5 min, the colour being stable for 24 h. All other measurements were made after for 10 min extracting.

Fe(III) chloride and ammonium thiocyanate were added in excess. Investigation showed that the absorbance increased up to a molar ratio of 20:1 for Fe(III)–diclofenac, and up to 8:1 for ammonium thiocyanate–Fe(III) chloride.

3.2. Effect of pH and ionic strength on complex formation

This study was carried out to determine the optimum pH at which the complex is formed. The variation of absorbance with the pH of the reaction mixture are represented in Fig. 2. The complex is only produced at pH values above 4.2. The general trend observed was a gradual increase in absorbance up to pH 5.8 to reach a plateau, followed by a steady decrease in absorbance after

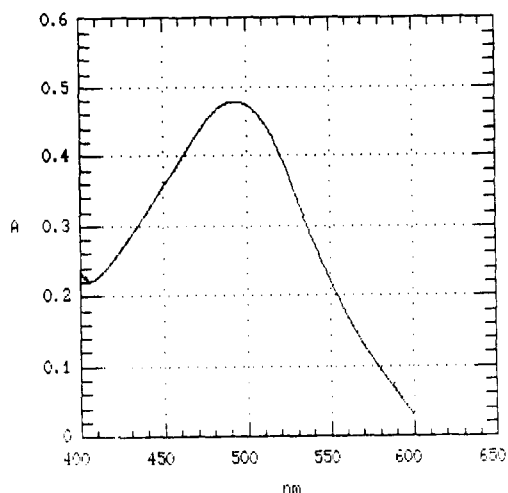


Fig. 1. Absorption spectra of diclofenac–Fe(III) complex in chloroform. Diclofenac concentration, 7.85×10^{-3} M; pH 6.0; $\mu = 0.19$ M.

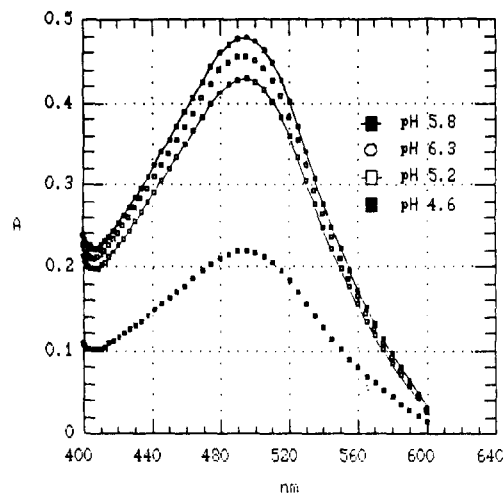


Fig. 2. The effect of pH on complex formation and extraction. Diclofenac concentration, 7.85×10^{-3} M; $[\text{Fe(III)}] = 3 \times 10^{-1}$ M; $\mu = 0.19$ M.

pH 6.2. Therefore the optimum conditions for complex formation were found in the pH range 5.8–6.2. As the shape of the absorption curves and positions of the absorption maximum do not vary with pH, it was assumed that in this pH range only one type of complex was formed.

The ionic strength also plays a significant role by its influence on the shape and intensity of the recorded peaks. The effect of ionic strength on complex formation was followed in the range 0.1–0.4 M. At an ionic strength of 0.2 M optimally shaped spectra were recorded.

3.3. Optimum conditions for complex formation

The absorbance and stability of the extracted complex can be maximised as a function of pH, supporting electrolyte concentration and concentration of colour reagents. In order to investigate the effect of each factor and their interactions a four variable, two level full factorial design was chosen. The variables were pH (*A*), ionic strength (*B*), concentration of Fe(III) chloride (*C*) and concentration of ammonium thiocyanate (*D*) in reaction mixture. The design matrix (Table 1) shows 16 treatment combinations of low (–) and high (+) level of the factors. From the estimate of factor effect (Table 2) it can be seen that each

Table 1
Matrix of experiments for the 2⁴ factorial design for diclofenac–Fe(III) complex

Trial	Factor level (low, –; high, +)				Absorbance
	A	B	C	D	
1	–	–	–	–	0.1332
2	+	–	–	–	0.2105
3	–	+	–	–	0.1476
4	+	+	–	–	0.2253
5	–	–	+	–	0.2254
6	+	–	+	–	0.3564
7	–	+	+	–	0.2306
8	+	+	+	–	0.3871
9	–	–	–	+	0.2085
10	+	–	–	+	0.4431
11	–	+	–	+	0.2997
12	+	+	–	+	0.4737
13	–	–	+	+	0.2338
14	+	–	+	+	0.3700
15	–	+	+	+	0.2550
16	+	+	+	+	0.4022

Low (–) and high (+) levels of the following factors:
Factor

		Value	
		Low (–)	High (+)
A	pH of reaction mixture	5.0	6.0
B	ionic strength (M)	0.1	0.2
C	Fe(III) chloride concentration (M)	0.05	0.10
D	ammonium thiocyanate concentration (M)	0.20	0.40

variable had a significant effect on the complex formation with the buffer exerting the most powerful effect. The interaction of the concentrations of Fe(III) chloride and concentration of ammonium thiocyanate was greater than the other combined effect which indicates that the amount of the formed complex influenced by the concentra-

tion of Fe(III) chloride is dependent on the concentration of ammonium thiocyanate.

In order to identify the optimum conditions for the analysis, a response surface design was chosen. Response surface can simultaneously represent two independent and one dependent variable. When all variables but four are held constant more than one response surface is required to represent the results. The response surface design is an effective means of locating the global optimum if a mathematical functional relationship between variables is known or can be assumed. For relationship between Fe(III) chloride concentration (x_1), ammonium thiocyanate concentration (x_2) and absorbance of extracted complex (A), while holding pH and ionic strength of reaction mixture constant (pH 6.0 and $\mu = 0.19$ M) model fitting methods give the equation:

Table 2
Estimates of factor effects and interactions

Average = 0.287631	$D = 0.096237$
$A = 0.141813$	$AD = 0.031187$
$B = 0.030038$	$BD = 0.013762$
$AB = -0.002963$	$ABD = -0.009437$
$C = 0.039863$	$CD = -0.080863$
$AC = 0.000912$	$ACD = -0.032212$
$BC = -0.007712$	$BCD = -0.009387$
$ABC = 0.012087$	$ABCD = 0.005812$

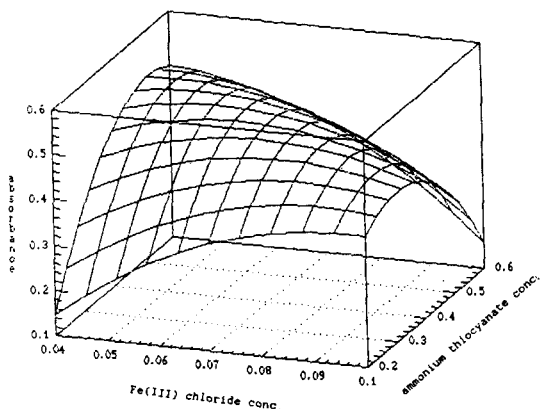


Fig. 3. The absorption response surface of diclofenac-Fe(III) complex in chloroform with respect to the Fe(III)-chloride concentration and NH_4SCN concentration. Diclofenac concentration, $7.85 \times 10^{-3} \text{ mol dm}^{-3}$; pH 6.0; $\mu = 0.19 \text{ mol dm}^{-3}$.

$$A = -1.0668 + 19.524x_1 + 4.3725x_2 - 77.34(x_1)^2 - 3.2475(x_2)^2 - 23.485x_1x_2$$

Maximum absorbance of extracted complex can be obtained with $6 \times 10^{-2} \text{ M}$ concentration of Fe(III) chloride and $4.8 \times 10^{-1} \text{ M}$ concentration of ammonium thiocyanate in reaction mixture as can be seen from assumed response surface shown in Fig. 3.

For the relationship between pH, ionic strength and absorbance while holding concentration of colour reagents constant at optimal concentrations, model fitting methods give the equation:

$$A = -5.3861 + 1.6916z_1 + 7.79204z_2 - 0.1228z_1^2 - 3.2784z_2^2 - 1.0854z_1z_2$$

The equation maximum is obtained at a pH (z_1) value of 6.08 and μ (z_2) = 0.185 M. The assumed response surface is shown in Fig. 4. All further measurements were carried out at pH 6.0 with an ionic strength 0.19 M and an eight-fold excess of ammonium thiocyanate concentration over that of Fe(III) chloride concentration.

3.4. Stoichiometry of the complex

The composition of the diclofenac-Fe(III) complex was determined by applying Job's

method of continuous variations. The concentration of aqueous diclofenac sodium and Fe(III) chloride solutions was 10^{-2} M . Nine solutions were used containing diclofenac sodium and ferric chloride in various molar ratios, so that their volume was constant at 5 ml with addition of 2.0 ml ammonium thiocyanate solution, 1.0 ml potassium chloride and 2.0 ml buffer solutions were prepared. The extraction was performed with 5 ml chloroform and the absorbance measured at 481 nm. The curve displays a maximum at a molar fraction $X_{\text{max}} = 0.33$ (Fig. 5), which indicates the formation of a 1:2 complex. The composition of the complex was confirmed by the molar ratio method. The curve obtained shows a break point at a molar ratio of 1:3 for Fe(III)-diclofenac.

3.5. Relative stability of the complex

The relative stability constant of the complex has been determined by applying the method of Sommer [16] on the basis of results obtained by Job's method for the composition of the complex and also by the application of Job's method of non-equimolar solutions [17]. By Job's method of non-equimolar solutions curves for five-fold and ten-fold excess of reagent were obtained (Fig. 6). The conditional stability constant was then calculated in the following way:

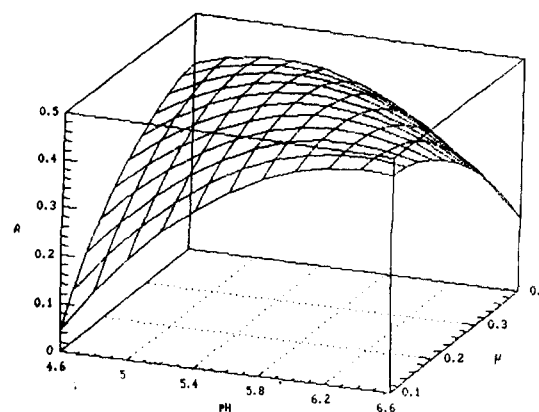


Fig. 4. The absorption response surface of diclofenac-Fe(III) complex in chloroform with respect to the pH and ionic strength of reaction mixture. Diclofenac concentration, $7.85 \times 10^{-3} \text{ M}$; $[\text{Fe(III)}] = 3 \times 10^{-1} \text{ M}$; pH 6.0; $[\text{NH}_4\text{SCN}] = 2.4 \text{ M}$.

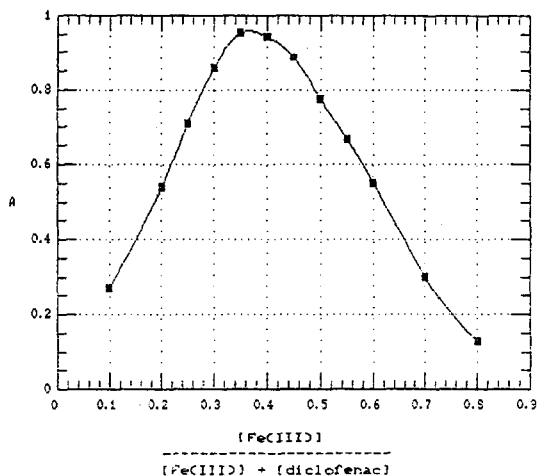


Fig. 5. Job's curve of equimolar solutions for the diclofenac-Fe(III) complex in chloroform. Diclofenac concentration, $[Fe(III)] = 10^{-2}$ M; pH 6.0; $\mu = 0.19$ M.

$$K' = \frac{(p-1)^2(2-3x_{\max})}{(c_{\text{diclofenac}})^2 p [(2+p)x_{\max} - 2]^3}$$

where $p = 5$ or 10 and x_{\max} is the projection of the peak maximum divided by the total volume of chloroform used for extraction in each case (12 ml). The values obtained by the two different methods are in good agreement (Table 3).

3.6. Validation

The method was tested for specificity, linearity,

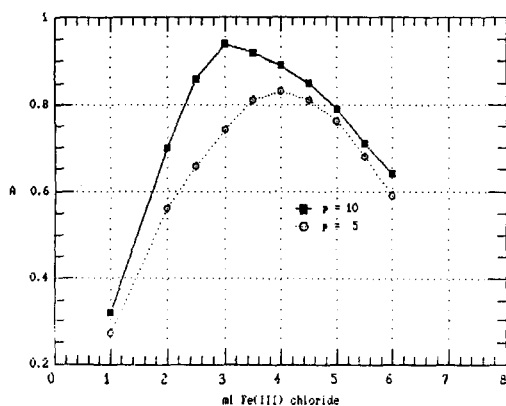


Fig. 6. Job's curve of non-equimolar solutions at 481 nm. Diclofenac concentration, 5×10^{-3} M; pH 6.0; $\mu = 0.19$ M.

Table 3

Conditional stability constant of the diclofenac-Fe(III) complex^a

Job's method of non-equimolar solutions^a

[Fe(III)]	p	X_{\max}	$\log K'$
2.5×10^{-2}	5	0.333	6.54
5×10^{-2}	10	0.208	6.56
		Mean:	6.55

Sommer's method^a

$\log K'$	$\log K'_{\min}$	$\log K'_{\max}$	S.D.	R.S.D. (%)
6.356	6.164	6.442	0.08	1.67

^a Conditions: pH 6.0; $\mu = 0.19$; $t = 25 \pm 0.5^\circ\text{C}$.

precision and reproducibility. The specificity of the method was investigated by observing any interference by other compounds from the tablet mass. Complete visible spectra of the diclofenac-Fe(III) complex with diclofenac obtained from sample solutions and standard solutions were obtained. In all cases the spectra were found to be identical. Although the effect of degradation products of diclofenac was not assessed, provided that this is less than 1% then this should not significantly affect the accuracy of the method.

The calibration graph used for the spectrophotometric determination of diclofenac showed that Beer's law is obeyed over the range 0.1–1.0 mg ml⁻¹: $A_{481} = a + bC$, where C is the concentration of diclofenac in mg ml⁻¹ (calculated in the final solution for extraction), $a = -0.00186$ and $b = 0.9774$; the regression coefficient $R' = 0.999$ for $n = 10$.

The precision of the method was checked at three different concentrations. The R.S.D. varied from 2.85 to 1.06% for concentrations of diclofenac sodium from 1.25–2.50 mg ml⁻¹.

The applicability of the proposed method for the assay of simple dosage forms was examined by analysing Toryxil[®] tablets. The recovery was 98.8% with R.S.D. of 1.52% ($n = 10$). The statistical data for the results obtained in the determination of diclofenac as a pure compound and in dosage forms are shown in Table 4.

Table 4
Recovery and R.S.D. data for diclofenac sodium in bulk drug and tablet forms

Sample ($n = 10$)	Concentration of solutions (mg ml ⁻¹)	Found (mg ml ⁻¹)	S.D. (μg)	R.S.D. (%)	S_v	Recovery (%)
Diclofenac sodium bulk drug	1.25	1.25	35.6	2.85	0.013	100.0
	250	2.50	24.3	0.97	0.009	100.0
	3.75	3.75	39.8	1.06	0.015	100.0
Toryxil* tablet (100 mg)	2.50	2.47	37.5	1.52	0.012	98.8

The colorimetric procedure, based on reaction of diclofenac sodium with Fe(III) chloride in the presence of ammonium thiocyanate and extraction of the complex formed is simple, rapid and quantitative. The limit of detection (SNR = 2) was 14.7 $\mu\text{g ml}^{-1}$. The method is suitable for the analysis of diclofenac sodium in commercial formulations.

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