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EXPERIMENTAL DESIGN APPLIED TO AN EXTRACTIVE SPECTROPHOTOMETRIC DETERMINATION OF DICLOFENAC

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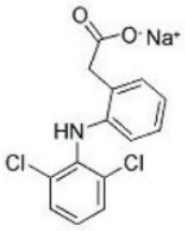
Abstract: A sensitive extractive-spectrophotometric method was developed for the determination of diclofenac. To obtain the optimal extractive-spectrophotometric response for diclofenac, two statistical models were applied: the Box-Behnken experimental design model and the response surface plot. Good agreement with Beer's law was found within the diclofenac concentration range of 0.2-2.0 μg/ml, with a detection limit of 0.02 μg/ml. The method was successfully applied to the determination of diclofenac in various pharmaceutical formulations, yielding satisfactory results.
Keywords: Diclofenac, extractive-spectrophotometric, Voltaren, experimental design.

INTRODUCTION

Diclofenac sodium (sodium [o-(2,6-dichloroanilino)-phenyl] acetate) is a synthetic, non-steroidal anti-inflammatory drug (NSAID) and analgesic agent, commonly used in the treatment of inflammatory and painful conditions of both rheumatic and non-rheumatic origin[1].



Structure of natrium diclofenac

Diclofenac is a potent inhibitor of cyclooxygenase, both in vitro and in vivo, thereby reducing the synthesis of prostaglandins, prostacyclin, and thromboxanes associated with the inflammatory process. Various analytical techniques have been employed for its determination.

Reported UV-Visible spectrophotometric methods for diclofenac sodium determination often suffer from low sensitivity or complexity. These methods include the use of chromogenic reactions[2-13].

On the other hand, experimental procedures in scientific research are often guided primarily by established laboratory protocols and subjective considerations of practicality. The effective application of statistical principles in experimental design ensures that experiments are conducted economically, efficiently, and that both individual and interaction effects can be evaluated.

To optimize the extractive-spectrophotometric response for diclofenac, this study applied two statistical models: the Box-Behnken experimental design model and the response surface plot[14]. Experimental design was used to evaluate the significance of parameters potentially affecting diclofenac extraction and their interactions. With the aid of response surface diagrams, the optimal extraction conditions were established. Diclofenac sodium was then analyzed using an extractive-spectrophotometric procedure, and the method was successfully applied to the determination of diclofenac sodium in pharmaceutical products.

EXPERIMENTAL

APPARATUS

A Spectronic 3000 Diode Array Milton Roy spectrophotometer, with 0.35 nm resolution, was used, coupled to a 486 PC and User Data version 2.01 (Milton Roy Inst. Co.) software for spectral data acquisition, storage, and manipulation. All data processing was carried out using a Hewlett-Packard Vectra 486/66. VL microcomputer equipped with the SGPlus software package, version 6.0 (Statgraphics).

REAGENTS

All reagents used were of analytical-reagent grade. Diclofenac sodium was obtained from Sigma. Acetic acid-sodium acetate buffer solutions (pH 2.50, 3.50, and 4.50) were prepared by mixing a 2 M sodium acetate solution with an appropriate volume of 1 M hydrochloric acid. Potassium chloride (from

Monterrey, S.A.), chloroform (from Baker Analyzed), and distilled-deionized water were used throughout. Pharmaceutical formulations containing diclofenac sodium were obtained from local drugstores and hospitals.

PROCEDURE

A freshly prepared 100 mg/l aqueous solution of diclofenac sodium was used as a standard solution. Aliquots of the diclofenac standard solution (containing between 20-200 μg) were placed in a series of 250 ml separating funnels. Then, 5.0 ml of buffer solution (pH 3.5) and 5.0 ml of 2.5 M KCl solution were added to each funnel. The total volume was adjusted to 100 ml with distilled-deionized water. For extraction, 10 ml portions of chloroform were added, and the funnels were shaken vigorously for 2 minutes. The two phases were allowed to separate, and the absorbance of the organic layer was measured at 278 nm against a reagent blank extracted under the same conditions.

DETERMINATION IN PHARMACEUTICAL FORMULATIONS

TABLETS

Five tablets were weighed and finely powdered in an agate mortar. Three accurately weighed portions, equivalent to one tablet each, were dissolved in the minimum volume of distilled-deionized water, sonicated for 5 minutes, filtered, and washed into a 100 ml calibrated flask. Suitable aliquots of this solution were analyzed following the procedure described above.

AMPOULES

The contents of five ampoules were mixed, shaken, and appropriate aliquots were transferred into a 100 ml calibrated flask, then diluted to volume with water. These aliquots were then treated as described above. Diclofenac content in the samples was determined using a calibration graph.

RESULTS AND DISCUSSION

The proposed method is based on the solubility of diclofenac in chloroform. Preliminary experiments with a number of organic solvents commonly used for solvent extraction showed that diclofenac had the highest solubility in chloroform. Chloroform is used to extract diclofenac from aqueous solutions increasing the selectivity of the determination. The optimum conditions for absorbance measurements in the extracted mixture of diclofenac-chloroform were then studied. Preliminar experiments suggested that the following parameters should be evaluated: pH, ionic strength and shaking time.

The effect of each variable parameter and their possible interactions were investigated using a Box-Behnken design (Table 1) which is another alternative to the 3k factorial design, the result is a design that makes efficient use of the experimental units and is also rotatable or nearly so, three-variable, three level design. For the extraction of diclofenac sodium, the dependent variable was the absorbance of the extracted at 278 nm measured in the organic phase after extraction. The independent variables were pH, shaking time and ionic strength. According to previous experience different levels of these variables were selected in order to maximize the information that could be extracted from the experimental data. The design matrix (Table 1) shows that fifteen trials should be carried out with the treatment combinations of low (-), medium (0) and high (+) levels of the variables while maintaining the amount of diclofenac constant. pH values of 2.5, 3.5 and 4.5, shaking time of 1, 2 and 3 minutes and an ionic strength of 0.125, 0.250 and 0.375 M were used for low, medium and high level respectively. Fifteen extracted Diclofenac-chloroform solutions were examined corresponding to the experimental matrix using the procedure described above.

From the statistical values for the effects of the variables (Tables 2 and 3), the Diclofenac extraction was found to be more sensitive to variations in the levels of pH and shaking time than to variations of ionic strength; the effect of pH and shaking time were the most significant. Response surface mapping is an effective way of locating the optimum if a mathematical relationship between the variables is known or can be assumed. The experimental data were fitted to a polynomial mathematical model so that the variables were adjusted until the calculated values were in close agreement with the experimental values. For the relationship between X= pH and Y = shaking time, model fitting methods gave the following polynomial equation:

$$A = 0.946333 - 0.059125\ X + 0.035125\ X - 0.04075\ XY - 0.365542\ X^2 - 0.018542\ Y^2.$$

The response surface map and the simpler form, the contour map of constant response (in two-dimensional space), are given in Figure 1 (a) and (b). The maximum response was obtained for a pH value of 3.5 with a shaking time of 2 minutes. These values were used as the optimum working conditions, the ionic strength was established to be in the Iow value.

The coefficient of multiple determination (R^2) is 0.9914 indícating that the variables explain the data perfectly. If the degrees of freedom are taken into account, the adjusted R^2 is 0.9760.

WAVELENGTH FOR ABSORBANCE MEASUREMENTS

The mixture between diclofenac-chlorofonn exhibits one maximum absorption band at 278 nm, where the blank absorbs negligibly, there fore, the wavelength of 278 nm was chosen for absorbance measurements (Figure 2).

ANALYTICAL CHARACTERISTICS

Using the optimum extractive-spectrophotometric conditions calibration graph was obtained. Good linearity was obtained in the concentration range of 0.2 to 2.0 μg/l of diclofenac (r = 0.9998). The precision of the method was 1.66 % evaluated by measuring replicate samples (n = 11, p = 0.05) containing 1.0 μg/l. Detection limit (L_D) calculated as the analyte concentration giving the signal corresponding to three times the standard deviation of the blank was 0.02 μg/l. The determination limit (L_Q) obtained as the analyte concentration giving the signals ten times higher than the standard deviation of the blank was 0.07 μg/l.

APPLICATIONS

The proposed method was used to determine diclofenac m vanous phannaceutical preparations. The results obtained are given in Table 4.

In the same pharmaceuticals, the recovery experiments were also carried out to provide further support for the validity of the proposed method. The results obtained for additions of diclofenac are shown in Table 4. In this table the mean percentage recoveries and their standard deviations are presented.

CONCLUSION

The proposed rnethod can be used successfully for the deterrnination of Diclofenac in pharrnaceutical sarnples. Selectivity of the rnethod is achieved through extraction with chlorofo rm. The proposed rnethod is more sensitive than other reported spectrophotornetric rnethods . The accuracy of the rnethod is good. The proposed rnethod is superior to other rnethods in terms of sirnplicity and convenience, and is therefore suitable for routine analysis.

	Coded	factor	levels	
No. of Factors	1	2	3	No. of Points.
3	±1	+1	o	4
	+ 1	0	±1	4
	0	+ l	+ 1	4
	0	0	0	3

Table 1. Box-Benhken Design.

Effect	Sum of Squares	d.f.	Mean Square	F-Ratio	p-value
A: pH	0.02796613	l	0.0279661	29.30	0.0029
B: shaking time	0.00987012	l	0.0098701	10.34	0.0236
C: Ionic strength	0.00125000	l	0.0012500	1.31	0.3043
AB	0.00664225	1	0.0066422	6.96	0.0461
AC	0.00883600	1	0.0088360	9.26	0.0287
BC	0.00220900	1	0.0022090	2.31	0.1887
AA	0.49336878	1	0.4933688	516.95	0.0000
BB	0.00126939	1	0.0012694	1.33	0.3009
CC	0.00004001	1	0.0000400	0.04	0.8479
Total error	0.00477192	5	0.0009544		
Total (corr.)	0.55743093	14			

R-Squared = 0.991439 R-Squared (adj. for d.f.) = 0.97603

Table 2. ANOVA for absorbance.

Variable(s)	Value
Average	0.946333 ± -0.017836
A: pH	-0.11825 ± -0.021845
B: shaking time	0.07025 ± -0.021845
C: ionic strength	-0.025 ± -0.02185
AB	-0.0815 ± -0.030893
AC	0.094 ± -0.030893
BC	0.047 ± -0.030893
AA	-0.731083 ± -0.032155
BB	-0.037083 ± -0.032155
ce	-0.006583 ± -0.032155

Table 3. Statistical values for the effect of the variables.
Standard error estimated from total error with 5 d.f. (t = 2.57141)

		Diclofenac recovery (%)[&]	
Trade name and source	Nominal diclofenac content	**Actual method**	**Standard addition method**
Volt.aren (Ciba-Geüzv)	75**	105.66 ± 1.03	104.54 ± 0.99
Votaren*retard(Ciba- Geigy)	100 ***	98.79 ± 2.78	
Nediclon	100 ***	104.14 ± 3.74	108.45 ± 1.76
Flogoken	100 **""	92.57 ± 1.76	
Cataflam (Ciba-Geigv)	50 *""*	99.06 ± 2.95	101.86 ± 1.47

Table 4. Determination of diclofenac* in some pharmaceutical preparations using the extractive spectrophotometric method.

*The active ingredient of all drugs in diclofenac sodium except for Cataflam which the active ingredient is diclofenac potassium.

[&]Average of 12 measurements

**mg (3 ml per ampoule)

***mg per tablet

Drug	Labeled arnount (mg)	Amount found (mg)+
Voltaren (Ciba-Oeigy)	75.00	79.24 ± 0.74
Votaren*retard(Ciba- Oeigy)	100.00	98.79 ±2.78
NedícJon	100.00	104.14 ± 1.76
Flogoken	100.00	92.57 ± 1.76
Cataflam (Ciba-Geigy)	100.00	99.06 ±1.47

Table 5. Determination of diclofenac* in some pharmaceutical preparations using the extractive spectrophotometric method.

* The active ingredient of all drugs is Diclofenac sodium except for Cataflam which the active ingredient is Diclofenac potassium.

+ Average of 12 measurements

CAPTIONS

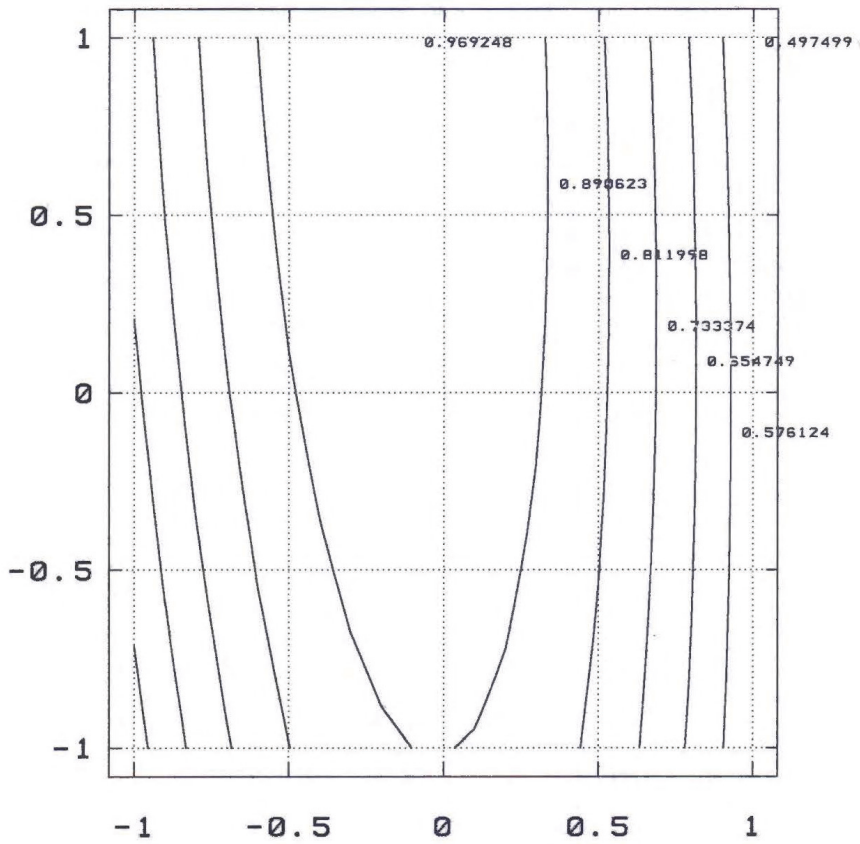


Figure 1.- Absorption response of Diclofenac in chloroform with respect to pH and shaking time (coded values). (a) Response surface map; and (b) contour map of constant response.

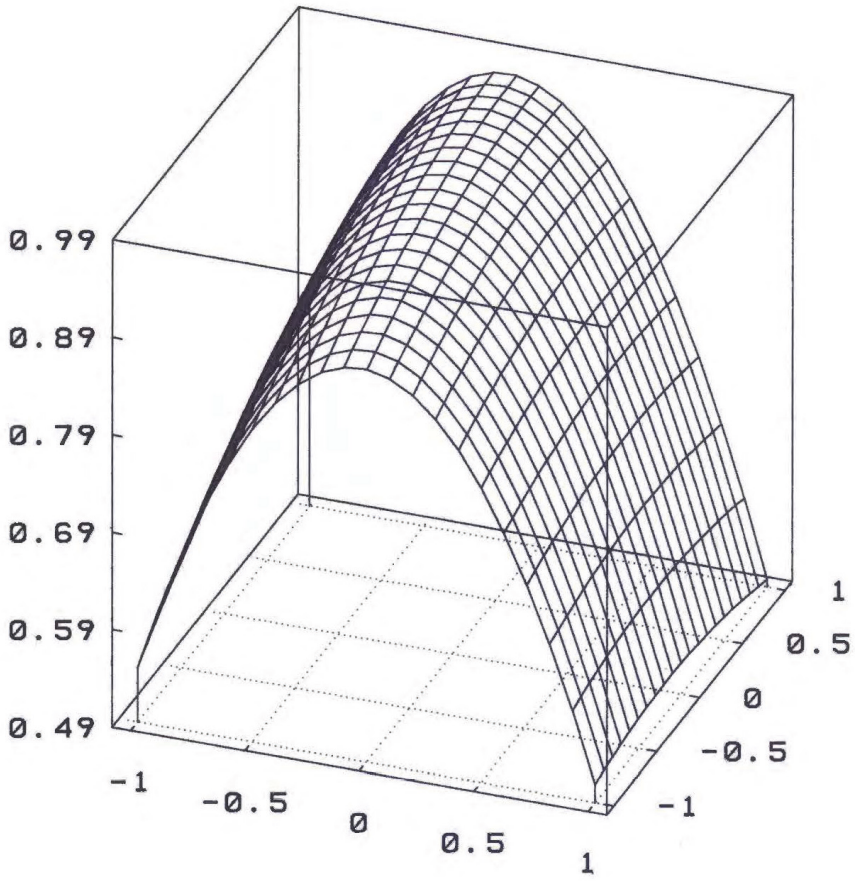


Figure 2.- Absorption spectrum of Diclofenac in chloroform. [Diclofenac sodium] = 6.3 x 10^{-3} mol/l; pH = 3.5, shaking time= 2 minutes and ionic strength = 0.125 mol/l.

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