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# Flow extraction spectrophotometric method for the determination of diclofenac sodium in pharmaceutical preparations

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**Abstract**

The spectrophotometric determination of trace amounts of diclofenac was carried out by liquid–liquid extraction using acridine yellow with a flow system. The determination of diclofenac sodium in the range of 3–80 μg $ml^{-1}$ was possible with a sampling frequency of 40 samples $h^{-1}$. The method was satisfactorily applied to the determination of diclofenac in pharmaceutical preparations. © 1997 Elsevier Science B.V.

*Keywords:* Diclofenac; Flow-injection method; Ion-pair extraction; Acridine Yellow; Spectrophotometry

## 1. Introduction

Diclofenac sodium, 2-[2,6-dichlorophenyl)-amino]benzeneacetic acid monosodium salt, is a nonsteroidal antiinflammatory agent with potent activity and outstanding tolerability in the treatment of rheumatic disease. Another therapeutic uses of the diclofenac are as analgesic and antipyretic. This phenylacetic acid derivative acts as a inhibitor of hyaluronidase, prostaglandins synthesis and platelet aggregation.

Several different methods have been reported for the determination of diclofenac including a variety of analytical techniques such as UV-visible spectrophotometry [1–7] spectrofluorimetry [8], gas and liquid chromatography [9–14], capillary electrophoresis [15], proton magnetic resonance spectrometry [16] and Raman spectroscopy [17]. An ion-selective electrode based on the diclofenac-nickel (II) bathophenantroline ion-pair complex as an ion-changer suite in a poly(vinyl chloride) matrix has been described for the determination of this drug [18]. Methods based upon the formation of ion-pairs and their extraction in organic solvents using methylene blue [19,20] and methylene violet [21] has also been used to determine diclofenac by molecular spectrometry.

There is a constant search for simple, reliable, automated and semiautomated methods for the rapid quantification of substances of therapeutic interest in pharmaceutical samples and biological fluids. However, it is worth noting that only one flow-injection method for the determination of diclofenac has so been described in the literature.

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This is based on its reaction with 2,4-dichloro-6-nitrophenol to generate a charge transfer complex which is monitored with an spectrophotometric detector set at 450 nm [22].

The purpose of this work was to investigate the formation and extraction behaviour of ion pairs of diclofenac with chromophores in order to develop a sensitive and automatic spectrophotometric method for its determination. Our results showed that acridine yellow offered the best possibilities of use in unsegmented flow configurations. The proposed method offers significant improvements over manual methods as regards safety, reagent consumption and throughput.

## 2. Experimental

### 2.1. Reagents

Diclofenac sodium was obtained from Sigma (St. Louis, MO, USA) and used as received. A standard solution was prepared by dissolving the drug in distilled water; this solution remained stable if kept refrigerated. Working solutions of lower concentrations were freshly prepared by appropriate dilution of the standard solution.

A $1 \times 10^{-3}$ M acridine yellow (2,7-dimethylacridine, C.I. 46 025) stock solution was prepared by dissolving the required amount of the dye (Sigma) in distilled water.

Stock solutions ($1 \times 10^{-3}$ M) of the dyes acridine orange, rhodamine 6G and rhodamine B, were prepared by dissolving the appropriate amounts of the product (Sigma) in water. Solutions of lower concentration were prepared by dilution with distilled water.

Phosphate buffers were prepared from 0.2 M potassium dihydrogen phosphate and sufficient 2 M hydrochloric acid or sodium hydroxide to give the desired pH. Buffers of the lower capacity were prepared by appropriate dilution with water. All solutions were prepared from analytical reagent grade materials in distilled water.

### 2.2. Apparatus

A Perkin-Elmer (Norwalk, CA, USA) 550 SE spectrophotometer was used for recording spectra, and a Pye-Unicam (Cambridge, UK) 8625 spectrophotometer was used as the detector in the flow system. A Gilson (Villiers le Bell, France) Minipuls HP4 peristaltic pump fitted with Tygon and Acidflex pump tubes and an Omnifit (Cambridge, UK) injection valve were also used.

### 2.3. Manifold

The configuration of the flow-injection manifold used (Fig. 1) has been described previously [23]. Phosphate buffer and acridine yellow solutions were pumped through Tygon tubes and chloroform was pumped through the Acidflex tube. The sample (120 μl) was introduced into the buffer stream by means of an Omnifit rotary valve to which a volume-control loop was attached. All connecting tubing was made of poly(tetrafluoroethylene) (PTFE). The absorbance of the organic phase was measured at 412 nm with a spectrophotometer equipped with a Hellma (Jamaica, NY, USA) 178.012 QS flow cell (18 μl inner volume and 10 mm light-path length) and was recorded with a Linseis (Selb, Germany) 6215 recorder.

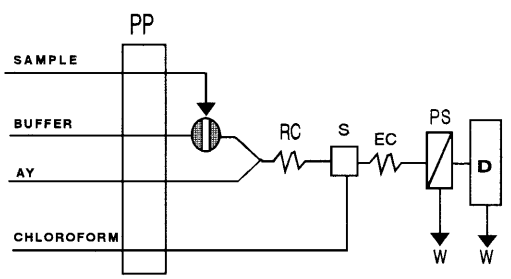


Fig. 1. Manifold for the determination of diclofenac sodium. PP, peristaltic pump; RC, reaction coil (30 cm × 0.5 mm I.D.); S, segmentor; PS, phase separator; EC, extraction coil (100 cm × 0.5 mm I.D.); W, waste; D, detector.

### 2.4. Determination of diclofenac in pharmaceutical preparations

The tablets were finely powdered and weighed. An amount of this powder, equivalent to about 100 mg of diclofenac was accurately weighed and shaken with 200 ml of distilled water in a water-bath at 50°C for 10 min. After cooling, the solution was filtered into a 1000 ml calibration flask, the residue was washed several times with water and the solution diluted with distilled water to the mark to obtain a solution of 100 μg $ml^{-1}$.

For the determination of the drugs in injections, the weight of the drug per ml was determined. A quantity equivalent to 25 mg of diclofenac was transferred to 250 ml volumetric flask and diluted to the mark with distilled water.

An appropriately diluted aliquot of these solutions was analysed using the standard additions method with multiple additions.

## 3. Results and discussion

Diclofenac can be transferred from the aqueous phase into the organic phase in the form of an ion pair with the cationic forms of the basic dyes. The extraction equilibria can be represented as follows:

$$DF^- + D^+ \leftrightarrow (DF^-\ D^+)$$

$$(DF^-\ D^+)_{aq} \leftrightarrow (DF^-\ D^+)_{org}$$

where $DF^-$ and $D^+$ denote the anion of the diclofenac and the protonated dyes, respectively, and the subscript *aq* and *org* refer to the aqueous and organic phases, respectively.

The dyes studied for diclofenac ion-pair formation were acridine yellow, acridine orange, rhodamine 6G and rhodamine B. Of the dyes tested, acridine yellow showed the greatest ion-pair extraction efficiency with the smallest reagent blank extraction.

The effect of the extracting solvent was also examined. The polarity of the solvent affects both the extraction efficiency and the absorbance. The results using acridine yellow are shown in Table 1, in which the response using chloroform was normalised as 100. In this study chloroform was the solvent selected.

Table 1
Effect of the extracting solvent on absorbance

| Solvent | $A_{\text{ion-pair}} - A_{\text{blank}}$ | Relative signal (%) |
|---|---|---|
| Chloroform | 0.470 | 100 |
| 1,2-Dichloroethane | 0.199 | 42.3 |
| Toluene | 0.012 | 2.6 |
| Ethyl acetate | 0.082 | 17.5 |
| Isobutyl methyl ketone | 0.194 | 41.2 |

[Diclofenac] $= 2.5 \times 10^{-5}$ M.
[Acridine yellow] $= 2.5 \times 10^{-4}$ M.

### 3.1. Extraction behaviour of diclofenac with acridine yellow

Both acridine yellow and its ion associate have identical spectra and so they must be separated if the ion pair is to be determined. The effect of the pH of the aqueous phase on the ion-pair extraction was studied using buffer solutions over the pH range 4.5–8. The absorbance of the chloroform extract was maximum and constant in the range 6.8–8.0.

The composition of the ion-pair was established by Job's method of continuous variations and by the molar ratio method using both a variable dye concentration and a variable diclofenac concentration. The result obtained with these methods showed that the composition of associate was equimolecular (1:1). The extraction constant for the above equilibrium was $\log K_{ex} = 5.9 \pm 0.2$.

Shaking times from 0.5–5 min did not produce any change in the absorbance, suggesting that equilibrium between the two phases in the extraction of the ion-pair can be attained rapidly. Reproducible absorbance readings at 438 nm were always obtained after a single extraction. The overall extraction efficiency was 96.4%.

### 3.2. Flow-injection determination of diclofenac

The above extraction behaviour suggested that diclofenac extracted with acridine yellow might

easily be used for the determination of the drug. The flow manifold for automation of the method is shown in Fig. 1.

### 3.3. Flow-injection variables

The variables studied were sample volume, lengths of the reaction coil and extraction coil and the flow rate in each reagent line. The concentration used in these experiments were as follows: buffer line 0.2 M phosphate buffer (pH 7.0); acridine yellow line $8 \times 10^{-3}$ M; and sample solution, $2 \times 10^{-5}$ M.

The volume of sample injected was varied between 35 and 245 μl. The peak height increased with increasing sample size up to 160 μl above which a double peak appeared. Therefore, a sample size of 160 μl was selected.

The reaction coil connects the valve and segmentor (see Fig. 1). Experiments were carried out in which this coil length was varied. A 30 cm × 0.5 mm I.D. coil was sufficient to yield the maximum signal because the ion-pair is formed rapidly. The effects of the extraction coil length on the peak height were also examined by varying the length up to 400 cm. As the peak height hardly altered with extraction coils more than 100 cm, a 100 cm (0.5 mm I.D.) extraction coil was adopted.

The selected flow rate of each stream is a compromise between sensitivity, peak resolution, phase separation, efficiency and rapidity of the analysis. Maximum absorbance values resulted in the adoption of flow rates of 1.8 ml $min^{-1}$ (0.9 ml $min^{-1}$ for each channel) and 2.0 ml $min^{-1}$ for the aqueous and organic streams, respectively.

### 3.4. Effect of reagent concentration

With the concentration of acridine yellow solution fixed at $8 \times 10^{-4}$ M, the buffer solution was varied over the pH range 4–8. The peak heights were maximum and constant above pH 6.8 (Fig. 2). A 0.2 M phosphate buffer (pH 7.0) was used as carrier. With this carrier, the peak heights increase with increasing acridine yellow concentration in the range $5 \times 10^{-4}$–$8 \times 10^{-4}$ M, but a further increase up to $10^{-3}$ M did not improve the sensitivity. Therefore, an acridine yellow concentration of $8 \times 10^{-4}$ M was selected.

### 3.5. Analytical features

The effect of the concentration of diclofenac on the absorbance was studied by measuring the peak height when 160 μl of diclofenac sodium solution of different concentrations were injected. The calibration graph was found to be linear between $8 \times 10^{-6}$–$2 \times 10^{-4}$ M and the regression equation obtained was:

$$A = (0.014 \pm 0.008) + (5667 \pm 52)\, C, \quad r = 0.9994$$

where $C$ is the molar concentration of diclofenac, $A$ is the absorbance and $r$ the correlation coefficient.

The relative standard deviations of ten injections of each solution containing $1.0 \times 10^{-5}$ and $1.0 \times 10^{-4}$ M of diclofenac were 1.36 and 0.88%, respectively. The limit of detection and quantitation (signals three and ten times the standard deviations of the average blank signal, respectively) were $1.3 \times 10^{-6}$ M and $4.4 \times 10^{-6}$ M, respectively. The sampling rate was 40 samples $h^{-1}$

The reproducibility of the method was studied by analysing, on five different days, ten identical solutions of diclofenac ($5 \times 10^{-5}$ M). Every day three injections of each solution were made; the relative standard deviation on the peak height was 2.34%.

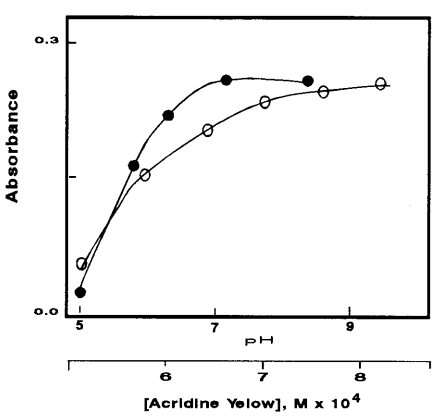


Fig. 2. Effect of pH (●) and acridine yellow concentrations (○) on the peak height.

Table 2
Tolerance to different species in the determination of diclofenac[a]

| Species added | Maximum tolerable molar ratio |
|---|---|
| Fructose, lactose, sucrose, propyleneglycol, starch, urea, benzyl alcohol | 100[b] |
| Sorbitol, maltose | 50 |
| Polyethylenglycol | 10 |
| Saccharin | 0.5 |

[a][Diclofenac] = 5 μg ml$^{-1}$.
[b]Maximum ratio tested.

### 3.6. Interferences

The influence of foreign substances that can commonly accompany diclofenac in pharmaceutical preparations was studied. Solutions of diclofenac and each compound tested were mixed to obtain samples containing 5.0 μg ml$^{-1}$ of the drug and up to 500 μg ml$^{-1}$ of the foreign compound. The tolerance ratio of each foreign compound was taken as the largest amount yielding an error of less than 3% in the analytical signal of the diclofenac. Table 2 shows the results obtained in this study.

### 3.7. Analysis of pharmaceutical preparations

The method was applied to the determination of diclofenac in commercially available pharmaceutical formulations. Interferences from the matrix were not a problem. The data in Table 3 show that the diclofenac contents were in good agreement with those obtained by the UV-spectrophotometric method [1]. The recoveries obtained by adding diclofenac sodium to each pharmaceutical formulation are shown in Table 4.

Table 3
Determination of diclofenac in pharmaceutical preparations

| Preparation | Supplier | Amount (mg) | |
|---|---|---|---|
| | | Claimed | Found[a] |
| Voltaren (ampoules) | Ciba Geigy | 75 | 78 ± 1.3 |
| Voltaren (tablets) | Ciba Geigy | 50 | 52 ± 2.0 |
| Dolotren (capsules retard) | Faes | 100 | 97.5 ± 2.0 |
| Luase (tablets) | Alfarma | 50 | 49 ± 1.0 |

[a] Mean of three determination ± standard deviation.

Table 4
Recovery of diclofenac added to pharmaceutical formulations

| Sample | Added (mg) | Found[a] (mg) | Recovery (%) |
|---|---|---|---|
| Voltaren (ampoules) | 10 | 9.8 | 98.0 |
| | 30 | 29.9 | 99.6 |
| | 60 | 59.2 | 98.6 |
| Voltaren (tablets) | 10 | 9.7 | 97.0 |
| | 20 | 19.7 | 98.5 |
| | 40 | 40.6 | 101.5 |
| Dolotren (capsules retard) | 30 | 29.2 | 97.3 |
| | 50 | 50.8 | 101.6 |
| | 100 | 98.4 | 98.4 |
| Luase (tablets) | 20 | 19.4 | 97.0 |
| | 40 | 40.7 | 101.7 |
| | 60 | 59.1 | 98.5 |

[a] Average of three determinations.

## 4. Conclusions

Results of the experiments with different dyes and extraction solvents showed that acridine yellow and chloroform were the most effective to be used in an unsegmented flow configuration with a continuous extraction system. This system overcome the complexity of normal extraction method and avoid problems and hazards involved in handling toxic organic solvents. The sensitivity, dynamic range and throughput of the method are good for the determination of diclofenac in pharmaceutical preparations.

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