Simultaneous potentiometric and fluorimetric determination of diclofenac in a sequential injection analysis system

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Abstract

Two independent methods for the determination of diclofenac were simultaneously implemented in an automated analytical system, based on the concept of sequential injection analysis, providing real-time assessment of results quality. The potentiometric detection was carried out with an ion-selective electrode based on a cyclodextrin while for the fluorimetric determination the sample was previously subject to in-line irradiation with UV light. The potentiometric and photochemical-fluorimetric determinations exhibited linear working ranges of $5 \times 10^{-6}$ to $1 \times 10^{-2}$ and $1 \times 10^{-6}$ to $1 \times 10^{-4}$ mol dm$^{-3}$, respectively. Relative standard errors of 0.5% for the potentiometric determination and 0.6% for the photochemical-fluorimetric determination were obtained after 10 consecutive injections of a $5 \times 10^{-5}$ mol dm$^{-3}$ diclofenac standard solution. The sampling rate was about 32 samples h$^{-1}$. Both methods were applied in the analysis of pharmaceutical formulations. The quality of results obtained was evaluated by comparison to the reference method, with no statistically significant differences for a 95% confidence level.

Keywords: SIA; Real-time accuracy assessment; Sodium diclofenac; Potentiometric determination; Photochemical-fluorimetric determination

1. Introduction

The importance of the chemical control of pharmaceutical products, as a result of the social, economic and public health impact that it imposes, demands the development of more sensitive, reliable, representative and responsive analytical methodologies, which can additionally accomplish large scale analysis with high sampling rates. In an effort to fulfill these requirements, automated methodologies based on distinct continuous flow techniques have been proposed [1], which offered the additional advantage of significant reductions of reagents consumption and waste generation. Among these techniques, sequential injection analysis (SIA) allows different analytical procedures to be accommodated in the same system without requiring its physical reconfiguration [2]. This feature has been explored to implement the concept of real time accuracy assessment in the quality control of pharmaceutical formulations, which is an aspect of great importance in routine laboratory analysis [3]. According to this concept, the sample is processed simultaneously by two quasi-independent methods being the results obtained intrinsically more accurate [4]. This work proposes the application of the concept of real time accuracy assessment in a SIA system for the control of diclofenac in pharmaceutical formulations.

Sodium diclofenac, sodium [o-(2,6-dichloroanilino)phenyl] acetate is a non-steroidal drug with...
anti-inflammatory, analgesic and antipyretic properties [5]. Due to its therapeutic relevance several methodologies have been proposed for the determination of diclofenac in pharmaceutical formulations including spectrophotometry [6–15], fluorimetry [16–19], potentiometry [20,21] chromatography [22–24] and nuclear magnetic resonance spectroscopy [25]. Among these methodologies, ion selective electrodes (ISE) are particularly advantageous since they are easily constructed in the laboratory at relatively low-cost, exhibit good analytical performance regarding selectivity and sensitivity and are robust and easily incorporated in continuous flow systems [26]. Similarly, the combination of photochemical reactions with fluorimetric detection in hydrodynamic systems has demonstrated to be a versatile, simple, selective and sensitive analytical strategy [27]. Taking advantage of the analytical potential of these two techniques, a SIA procedure combining a new diclofenac selective sensor for potentiometric detection and the fluorimetric determination following in-line sample irradiation with UV light, is proposed.

2. Experimental

2.1. Reagents and solutions

All solutions were prepared with deionized water (conductivity < 0.1 μS cm⁻¹) and analytical grade chemicals without further purification were used. Electrode membranes were prepared with α-cyclo-dextrin (Sigma-C-4642) as neutral carrier, tetradodecylammonium bromide (Fluka-87249) as fixed cationic site, 2-nitrophenyloctyl ether (Fluka-73732) as mediator solvent and PVC (Fluka-81392) as immobilising matrix. The solid polymers were dissolved in tetrahydrofuran (Merck-1.09731).

To enable the simultaneous adjustment of pH and ionic strength an 0.2 mol dm⁻³ ammonia-ammonium sulphate buffer solution adjusted to pH 9.9 and I = 0.1 mol dm⁻³ was used as carrier solution. The stock sodium diclofenac (Sigma-D-6899) solution (1 × 10⁻² mol dm⁻³) was prepared by rigorous weighing of the solid and dilution with the buffer solution. The less concentrated solutions were obtained by rigorous dilution of the concentrated ones with the pH and ionic strength adjusting solution. In the trials carried out by HPLC according to the method proposed by USP [28], which was used as the reference method, the mobile phase was composed of a mixture of methanol and phosphate buffer pH 2.5 (70:30) filtered and degassed. In this analysis, a 0.75 mg/ml diclofenac standard solution was previously diluted with in a diluent solution composed of a 70:30 mixture of methanol and water.

2.2. Sample preparation

The preparation of the solid sample solutions (tablets and suppositories) comprised the homogenisation of 20 tablets or suppositories, after which an amount of the resulting powder or paste equivalent to 16 mg diclofenac was rigorously weighed and dissolved in 50.0 ml of buffer solution. This solution was later filtered and diluted with the same buffer in such a way as to obtain a solution whose expected diclofenac concentration was 5 × 10⁻⁵ mol dm⁻³. The liquid sample solutions (injectables) were prepared, after prior homogenisation of the liquid contained in five ampules, by diluting an appropriate sample aliquot with buffer solution in such a way as to obtain a diclofenac concentration similar to the above mentioned one.

The preparation of tablet and suppository sample solutions according to the reference procedure [28], involved the ultrasonic dissolution with diluent solution of a sample amount corresponding to a final diclofenac concentration of 0.75 mg/ml, for 30 min. These solutions were subsequently filtered through a 0.5 μm porosity filter and used with no other treatment. For injectable formulations, an aliquot from the mixed content of five ampules was diluted with diluent solution, in such a way as to obtain a 0.75 mg/ml sodium diclofenac concentration. This solution was filtered prior to analysis.

2.3. Construction of conventional and tubular electrodes

The polymeric membranes used in the construction of electrodes were prepared by dissolving α-cyclodextrin (1.2% w/w) and tetradeclammonium bromide (0.4% w/w) in the mediator solvent, 2-nitrophenyloctyl ether (65.6% w/w). Thereafter, the PVC (32.8% w/w) was dissolved in tetrahydrofuran and added to the previous mixture.
Diclofenac-selective electrodes, without reference solution, were constructed in both conventional and tubular configurations following the methodology previously described [29]. The membrane solutions were directly applied on the conductor support, made up of a mixture of epoxy resin (Araldite) with graphite powder [30]. Following preparation of the electrodes, they were put on hold until the tetrahydrofuran was completely evaporated and thereafter placed in a $1 \times 10^{-4}$ mol dm$^{-3}$ sodium diclofenac solution to condition the membrane.

2.4. Apparatus

A Crison micropH, model 2002 decimillivoltameter (sensitivity $\pm 0.1$ mV) was used to measure the potential differences between a Russel model 90-0029 double-junction AgCl/Ag reference electrode and the indicator electrode. The outer compartment of the reference electrode contained an ammonia–ammonium buffer solution (pH 9.9 and $I = 0.1$ mol dm$^{-3}$). The pH measurements were performed with a Philips GAH 110 glass electrode.

To carry out the fluorescence measurements a Gilson model 121 fluorimeter, equipped with a 305–395 nm Gilson 095312 excitation filter, a 430–470 nm Gilson 095442 emission filter and a 9/$\mu$H9262 l optical volume flow cell, was used. The irradiation, at 254 nm, was accomplished with a Philips TUV 15 W/G15T8 low pressure mercury lamp. The photo-degradation reactor was implemented by coiling a PTFE tubing (200 cm length and 0.8 mm i.d.) around the lamp, which was subsequently placed inside a protecting chamber equipped with a fan to cool the reactor to room temperature. For the same reason, the lamp was only switched on when the sample was in the interior of the photo-reactor.

The determinations by sequential injection analysis were carried out with the multi-task flow-system (Fig. 1), whose mode of functioning was previously described [31]. This was composed of a Gilson Minipuls 3 peristaltic pump (Villier-le-Bell, France), equipped with a PVC propulsion tube of the same brand (1.85 mm i.d.); a six-port rotary valve (RV) from Valco Instruments, model Cheminert C15-3186E (Houston, USA), and a NResearch 161 T031 (Stow, USA) three-way solenoid valve (SV). The different components of the manifold were interconnected with PTFE tubing of 0.8 mm i.d. Some homemade devices such as joint pieces, grounding electrode, supports for tubular and reference electrodes as described elsewhere were also used [32]. The system was computer-controlled through an Advantech PCL 711B interface card, and the control and data acquisition and processing program was developed in QuickBasic language.

Sample analysis by the USP method [28] was carried out in a chromatographic Merck Hitachi system, made up of a pump (model 7100), connected to an Rhododyne injector model 7725i (20/$\mu$l loop) and a Waters X Terra$^{TM}$ RP 8 column (3.9 mm $\times$ 150 mm), packed with 5 $\mu$m beads, and a model 7455 diode-array detector. Data were processed using the same brand software, model D7000.

2.5. Procedures

In the multi-task system (Fig. 1), the holding coil (HC) and the transmission channels between RV and the detection systems were initially filled up with carrier/ionic strength adjuster solution by selecting ports 2/4 and placing the peristaltic pump in propulsion mode. This mode of operation was maintained until a stable baseline was obtained in the two detectors. The instructions required for an analytical determination cycle of diclofenac are defined in Table 1. The first three steps of the calibration procedure and the first two steps of the measurement procedures correspond to the alteration of the chemical nature of the solutions that access the system through the SV. The selection of port 1 (first step of calibration cycle) with the SV activated, enabled the filling up of the active access channel with a $1 \times 10^{-4}$ mol dm$^{-3}$ sodium diclofenac solution for both the potentiometric and fluorimetric calibration procedures. When the SV was not activated, the passive port channel was filled up with carrier solution for the calibration procedure or with sample solution in the case of analytical determinations.

The potentiometric and fluorimetric calibration procedures were carried out by preparing in-line calibration solutions of different concentration [31]. For this effect, the last two steps of the calibration cycle, involving RV ports 1 and 2/4, were sequentially executed four times. Calibration solutions of respective diclofenac concentration $2.5 \times 10^{-7}$, $5 \times 10^{-7}$, $7.5 \times 10^{-7}$ and $1 \times 10^{-8}$ mol dm$^{-3}$ were prepared.
automatically in the interior of the system for each selection of port 1 by means of the SV on/off cycles. Small standard diclofenac plugs \((1 \times 10^{-4} \text{ mol dm}^{-3})\) were aspirated into the HC by activation of SV (SV on) in an alternating mode with plugs of carrier solution when the SV was in the off position (SV off). The mutual dispersion between both solutions during the aspiration stage and during the sending to the detector.

Table 1

<table>
<thead>
<tr>
<th>System operation</th>
<th>RV SV state(^a)</th>
<th>L state</th>
<th>Volume (μl)</th>
<th>Flow-rate (ml/min)</th>
<th>Flow direction</th>
<th>Event</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diclofenac selective electrode calibration</td>
<td>1 On</td>
<td>–</td>
<td>200</td>
<td>1.5</td>
<td>R</td>
<td>Stock diclofenac solution</td>
</tr>
<tr>
<td></td>
<td>1 Off</td>
<td>–</td>
<td>200</td>
<td>1.5</td>
<td>R</td>
<td>Carrier</td>
</tr>
<tr>
<td></td>
<td>5 –</td>
<td>–</td>
<td>600</td>
<td>4.0</td>
<td>F</td>
<td>Waste</td>
</tr>
<tr>
<td></td>
<td>1 On 25, 50, 75 and 100%</td>
<td>–</td>
<td>400</td>
<td>2.4</td>
<td>R</td>
<td>In-line diclofenac standard preparation</td>
</tr>
<tr>
<td></td>
<td>2 –</td>
<td>–</td>
<td>13000</td>
<td>7.9</td>
<td>F</td>
<td>Diclofenac measurement</td>
</tr>
<tr>
<td>Diclofenac potentiometric determination</td>
<td>1 Off</td>
<td>–</td>
<td>200</td>
<td>1.5</td>
<td>R</td>
<td>New sample</td>
</tr>
<tr>
<td></td>
<td>5 –</td>
<td>–</td>
<td>400</td>
<td>4.0</td>
<td>F</td>
<td>Waste</td>
</tr>
<tr>
<td></td>
<td>1 Off</td>
<td>–</td>
<td>400</td>
<td>2.4</td>
<td>R</td>
<td>Diclofenac sample volume</td>
</tr>
<tr>
<td></td>
<td>2 –</td>
<td>–</td>
<td>13000</td>
<td>7.9</td>
<td>F</td>
<td>Diclofenac measurement</td>
</tr>
<tr>
<td>Diclofenac fluorimetric calibration</td>
<td>1 On</td>
<td>–</td>
<td>200</td>
<td>1.5</td>
<td>R</td>
<td>Stock diclofenac solution</td>
</tr>
<tr>
<td></td>
<td>1 Off</td>
<td>–</td>
<td>200</td>
<td>1.5</td>
<td>R</td>
<td>Carrier</td>
</tr>
<tr>
<td></td>
<td>5 –</td>
<td>–</td>
<td>600</td>
<td>4.0</td>
<td>F</td>
<td>Waste</td>
</tr>
<tr>
<td></td>
<td>1 On 25, 50, 75 and 100%</td>
<td>–</td>
<td>400</td>
<td>2.4</td>
<td>R</td>
<td>In-line diclofenac standard preparation</td>
</tr>
<tr>
<td></td>
<td>4 –</td>
<td>On –</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>Diclofenac irradiation during 15 s</td>
</tr>
<tr>
<td></td>
<td>4 –</td>
<td>Off 2330</td>
<td>2.0</td>
<td>F</td>
<td>Diclofenac measurement</td>
<td></td>
</tr>
<tr>
<td>Diclofenac fluorimetric determination</td>
<td>1 Off</td>
<td>–</td>
<td>200</td>
<td>1.5</td>
<td>R</td>
<td>New sample</td>
</tr>
<tr>
<td></td>
<td>5 –</td>
<td>Off 400</td>
<td>4.0</td>
<td>F</td>
<td>Waste</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1 Off</td>
<td>–</td>
<td>400</td>
<td>2.4</td>
<td>R</td>
<td>Diclofenac sample volume</td>
</tr>
<tr>
<td></td>
<td>4 –</td>
<td>Off 780</td>
<td>2.6</td>
<td>F</td>
<td>Diclofenac in the photoreactor</td>
<td></td>
</tr>
<tr>
<td></td>
<td>4 –</td>
<td>On –</td>
<td>–</td>
<td>F</td>
<td>Diclofenac irradiation during 15 s</td>
<td></td>
</tr>
<tr>
<td></td>
<td>4 –</td>
<td>Off 2330</td>
<td>2.0</td>
<td>F</td>
<td>Diclofenac measurement</td>
<td></td>
</tr>
</tbody>
</table>

Steps for diclofenac determination in pharmaceutical formulations with the SIA system in Fig. 1: RV: rotary valve; SV: solenoid valve; L: UV lamp; R: reverse; F: forward.

\(^a\) Each SV cycle as 2 s.
enable the simulation of the intercalation of the different standard solutions with the above mentioned concentrations. To obtain the four standards, the SV was activated during 25, 50, 75 and 100% of each on/off cycle time applied to the SV.

For the diclofenac ISE calibration, 400 H9262 l of solution (corresponding to 10 cycles of the SV) were aspirated and sent to the detector at a flow rate of 7.9 ml min−1. For the analytical determinations, port 1 was selected and filled up with the sample solution to be measured after which 400 H9262 l of sample were aspirated to the HC and sent to the detector (port 2) with the same flow rate. For the fluorimetric calibration, 400 H9262 l of solution (corresponding to 10 cycles) were aspirated and sent to the photoreactor. When the sample plug reached the photoreactor, the flow stopped and the sample was irradiated for 15 s, after which it was sent to the detector (port 4) at a flow rate of 2.0 ml min−1. For the analytical determinations, port 1 was selected and filled up with the sample solution to be measured. Following this, 400 H9262 l of sample were aspirated to the irradiation system with fluorimetric detection (port 4) under exactly the same conditions as those previously described.

3. Results and discussion

3.1. Potentiometric determination

The electrode membrane was based on the use of a cyclodextrin that forms inclusion compounds with diclofenac 1:1 [19]. The cyclodextrins are cyclical oligosaccharides with torus-like form, consisting of 6–12 glucose units with an α-α (1–4) linkage, which could form inclusion compounds with several organic molecules of adequate size and polarity. Recently, they have been incorporated in elasticised PVC membranes and used as ionophores in ISEs for the detection of a wide range of alkyl and arylammonium ions, with other electrodes based on cyclodextrins with enantioselectivity being reported in the literature [33]. Based on this knowledge, the construction of a diclofenac selective sensor was proposed, in a first stage with conventional configuration and lately with a tubular configuration, for application in the automated analysis in a multitask flow system.

The general working characteristics of conventional electrodes were evaluated by performing regular calibrations with sodium diclofenac solutions within the concentration range of 5 × 10−7 to 1 × 10−2 mol dm−3, in solutions with adjusted pH and ionic strength and according to the IUPAC recommendations [34]. The electrodes presented good working characteristics namely a wide range of linear response (4 × 10−6 to 1 × 10−2 mol dm−3) with an anionic slope of 57.5 mV per decade, a practical limit of detection of 1 × 10−6 mol dm−3, good reproducibility (0.4 mV per day) and a rapid response time (<20 s). To determine the characteristics of the electrodes in fixed pH solutions, the influence of this parameter on the response of the electrodes was first studied, in solutions of 5 × 10−4 and 5 × 10−3 mol dm−3 sodium diclofenac. The different pH conditions were obtained by addition of small volumes of concentrated potassium hydroxide and sulphuric acid solutions. The operational pH range was obtained when the potential values did not vary by more than ±5 mV. As can be observed in Fig. 2, in the range between 8.2 and 12.7 pH units, the electrodes’ response is practically constant and independent of pH, since diclofenac was completely ionised in this pH zone. In the pH zones <8.2, there was a precipitation of diclofenac in the form of acid, by protonation of the secondary amine. Also the interference from the H+ ion causes an oscillation in potential. Considering this behaviour, a buffer solution of ammonia-ammonium sulphate (pH 9.9) was used in the characterisation trials of the electrodes. This solution acted simultaneously as an adjuster of both ionic strength and pH.
The extension of interference of some organic and inorganic anions was evaluated determining the coefficients of potentiometric selectivity (log $K_{POT}$) by the separated solutions method [34] with the interferent and primary ion at various concentration levels. The sequence of interferents obtained for the units constructed was citrate $<$ phosphate $<$ tartarate $<$ borate $<$ aminobenzoate $<$ chloride $<$ benzoate $<$ acetate $<$ aminosalicylate $<$ nitrate $<$ salicylate. The diagram represented in Fig. 3, when the concentration of interferent and primary ion was $5 \times 10^{-3}$ mol dm$^{-3}$, showed that the electrodes constructed did not present significant interferences from the ions studied. It is equally important to refer that the level of interference is possibly related to the charge, hydrophilicity and size of the hydrated ions, which determine its inclusion in the cyclodextrin cavities [35]. No adverse effect on the response of the electrode was observed for up to a 100-fold excess of many pharmaceutical excipients and diluents commonly used for drug formulations such as fructose, glucose, lactose, mannitol, starch and carboxymethylcellulose.

The general working characteristics of the tubular detectors were evaluated after coupling it to the SIA system (Fig. 1) and using an ammonia-ammonium sulphate buffer solution (pH 9.9 and $I = 0.1$ mol dm$^{-3}$) as carrier solution. An optimum injection volume of 400 µl was selected at a flow rate of 7.9 ml min$^{-1}$ to the detector since this conditions enabled the attainment of an analytic signal of comparable intensity to that obtained by the injection of larger volumes. Under these flow conditions the characteristics of ISEs were determined (Table 2). The units presented a Nernstian response in the concentration interval $5 \times 10^{-6}$ to $1 \times 10^{-2}$ mol dm$^{-3}$, with a slope of $-56.6 \pm 1.0$ mV per decade. Relative standard errors of 0.93 and 0.53% were obtained after 10 consecutive injections of two standard solutions of sodium diclofenac of concentrations of $1 \times 10^{-5}$ and $5 \times 10^{-3}$ mol dm$^{-3}$, respectively. The sampling rate was samples per hour.

Having optimised the flow conditions and known the characteristics of the ISEs, the automation of both the in-line ISEs calibration procedure and sample
Table 2

Analytical features of merit of the developed procedures for the determination of diclofenac

<table>
<thead>
<tr>
<th></th>
<th>Potentiometric procedure</th>
<th>Photochemical-fluorimetric procedure</th>
</tr>
</thead>
<tbody>
<tr>
<td>Linear range (mol dm$^{-3}$)</td>
<td>5 $\times$ 10$^{-6}$ to 1 $\times$ 10$^{-2}$</td>
<td>1 $\times$ 10$^{-6}$ to 1 $\times$ 10$^{-4}$</td>
</tr>
<tr>
<td>Detection limit (mol dm$^{-3}$)</td>
<td>2 $\times$ 10$^{-6}$</td>
<td>6 $\times$ 10$^{-3}$</td>
</tr>
<tr>
<td>Practical limit of detection (mol dm$^{-3}$)</td>
<td>2 $\times$ 10$^{-6}$</td>
<td>2 $\times$ 10$^{-4}$</td>
</tr>
<tr>
<td>Lower limit of linear range (mol dm$^{-3}$)</td>
<td>5 $\times$ 10$^{-6}$</td>
<td>1 $\times$ 10$^{-5}$</td>
</tr>
<tr>
<td>Regression equation</td>
<td>$mV = 56.63 \log C + 360.91$</td>
<td>$\log F = 0.9995$</td>
</tr>
<tr>
<td>Quadratic correlation coefficient ($R^2$)</td>
<td>0.9992</td>
<td>0.9995</td>
</tr>
<tr>
<td>R.S.D. (%)</td>
<td>0.83; 0.53</td>
<td>1.82; 0.64</td>
</tr>
<tr>
<td>Sampling rate (samples per hour)</td>
<td>33</td>
<td>32</td>
</tr>
</tbody>
</table>

$^1$ Practical limit of detection determined according to IUPAC recommendations [34].
$^2$ Lower limit of detection determined according to IUPAC recommendations.
$^3$ $mV$: potentiometric signal.
$^4$ $\log F$: logarithm of fluorescence signal; R.S.D.: relative standard deviation for 10 replicates of a 1 $\times$ 10$^{-5}$ and a 5 $\times$ 10$^{-5}$ mol dm$^{-3}$ diclofenac standard solution.

Determination, was carried out. As previously demonstrated [31] the system enable the in-line preparation of calibration solutions from the stock solution using the SV placed in port 1. By means of successive momentary activations of SV during the aspiration through port 1, it was possible to create a stack of small zones of sodium diclofenac stock solution and ionic strength adjuster solution. The aspiration and subsequent flow reversal contributed to the homogenisation of sodium diclofenac concentration over all segments, with the final concentration being determined by the proportion of time during which the SV remains activated. For injection volumes of 400 µl (10 cycles) of sodium diclofenac solutions, analytical signals with an intensity of 95% comparatively to those obtained with the conventional analysis of the same calibration solutions, were obtained. Equally, when the calibration carried out with the 2.5 $\times$ 10$^{-3}$, 5 $\times$ 10$^{-3}$, 7.5 $\times$ 10$^{-3}$ and 1 $\times$ 10$^{-2}$ mol dm$^{-3}$ diclofenac standards prepared within the flow system was compared with the calibration carried out with standards prepared manually no difference in the intensity of the obtained analytical signals was observed.

3.2. Photochemical-fluorimetric determination

Diclofenac in its native form presents an excitation maximum at a wavelength of 279 nm and a Stokes-shift of 73 nm [17]. Preliminary batch experiments to evaluate the effect of direct UV light irradiation in diclofenac solutions revealed the formation of photoproducts with increased quantum yields, even with small irradiation times. Irradiation for 30 s of a M mol dm$^{-3}$ diclofenac solution generates a new emission band with a maximum fluorescence of 420 nm, and with an intensity 485 times greater when compared with the diclofenac fluorescence band in the native form (Fig. 4). This behaviour is attributed to the formation of a photoproduct that yields a much more intense fluorescence than that of diclofenac native one.

In the flow studies, it was verified that the irradiation time, volume of sample injected, flow rate and the reaction pH, produced significant differences in the intensity of fluorescence signals. The sample residence time in the photoreactor was the most critical parameter and its effect was evaluated by filling up the reaction coil (RC) with reproducible sample volumes, stopping the flow and placing the lamp in the ON position over increasing time periods. The results obtained indicated that the intensity of fluorescence attained a maximum when the sample was irradiated for a period of 10–30 s. For irradiation times >30 s a decrease in fluorescence was observed. The sample injection volume and flow rate were also studied. A volume of 400 µl and a flow rate of 2.0 ml min$^{-1}$ was selected for posterior experiments as they represented the conditions that corresponded to the best compromise between sampling frequency and signal intensity. The effect of reaction pH was studied by injecting...
a 5 × 10⁻⁵ mol dm⁻³ solution prepared in HCl–KCl (pH 2), citric acid–sodium citrate (pH 3, 4, 5, 6); KH₂PO₄–K₂HPO₄ (pH 7), ammonia–ammonium sulphate (pH 8, 9, 10, 11) buffer solutions, while using the same buffers as carriers solutions. The intensity of obtained signals was higher at alkaline pH and constant at pH > 9, demonstrating that this parameter has a major influence in the reaction kinetic. Accordingly, in the following experiments likewise what happened with the potentiometric procedure an ammonia–ammonium buffer of fixed pH (pH 9.9) was used as carrier solution. Other analytical figures of merit for the determination of diclofenac are presented in Table 2. These data was obtained after carrying out at least three measurements at distinct concentration levels and by using the previously fixed

Table 3

Obtained results of diclofenac pharmaceuticals formulations (mg diclofenac/unit)

<table>
<thead>
<tr>
<th>Samples</th>
<th>Reference method</th>
<th>Potentiometric determination</th>
<th>R.D. (%)</th>
<th>Photochemical-fluorimetric determination</th>
<th>R.D. (%)</th>
<th>Average results</th>
<th>R.D. (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>FenilV® dispersible</td>
<td>46.9 ± 0.3</td>
<td>46.5 ± 0.9</td>
<td>−0.9</td>
<td>46.4 ± 0.3</td>
<td>−1.0</td>
<td>46.5 ± 0.9</td>
<td>−0.9</td>
</tr>
<tr>
<td>Diclofen® 50</td>
<td>51.6 ± 0.9</td>
<td>52 ± 1</td>
<td>0.2</td>
<td>52.4 ± 0.3</td>
<td>1.0</td>
<td>52 ± 1</td>
<td>0.2</td>
</tr>
<tr>
<td>Diclofenac merck® 50</td>
<td>49.6 ± 0.3</td>
<td>50 ± 1</td>
<td>0.8</td>
<td>50.7 ± 0.3</td>
<td>2.2</td>
<td>50 ± 1</td>
<td>0.8</td>
</tr>
<tr>
<td>Voltaren® retard 100</td>
<td>99.3 ± 0.4</td>
<td>99.9 ± 0.7</td>
<td>0.6</td>
<td>100.6 ± 0.3</td>
<td>1.3</td>
<td>100.2 ± 0.9</td>
<td>0.9</td>
</tr>
<tr>
<td>Fenil® V 75 mg/2 ml</td>
<td>74.7 ± 0.5</td>
<td>74 ± 1</td>
<td>−0.9</td>
<td>75.4 ± 0.5</td>
<td>0.9</td>
<td>75 ± 1</td>
<td>0.4</td>
</tr>
<tr>
<td>Voltaren® suppository 120</td>
<td>74.7 ± 0.5</td>
<td>75 ± 1</td>
<td>0.4</td>
<td>74.5 ± 0.5</td>
<td>−0.3</td>
<td>75 ± 1</td>
<td>0.4</td>
</tr>
<tr>
<td>Flumetyl® suppository 100</td>
<td>99.6 ± 0.5</td>
<td>99.8 ± 0.2</td>
<td>0.2</td>
<td>99.5 ± 0.6</td>
<td>−0.1</td>
<td>99.7 ± 0.1</td>
<td>0.1</td>
</tr>
</tbody>
</table>

a Average of four determinations ± S.D.

b Relative deviation to the reference method.
hydrodynamic conditions. The calibration curves obtained showed a linear response in a concentration interval of two-orders of magnitude between $1 \times 10^{-6}$ and $1 \times 10^{-4}$ mol dm$^{-3}$. The repeatability of the described procedure was calculated by injecting diclofenac solutions of $1 \times 10^{-5}$ and $5 \times 10^{-5}$ mol dm$^{-3}$ and carrying out 10 replicates. The influence of compounds that can be found in pharmaceutical formulations was studied by preparing diclofenac solutions containing a quantity of the potential interferent for up to 100-fold regarding diclofenac concentration. No interferences were detected from the excipients used in the manufacture of the studied formulations.

3.3. Analytical control of pharmaceutical formulations

Sodium diclofenac determinations were performed in various pharmaceutical formulations by the two proposed methodologies. The instructions required for the analytical cycle of diclofenac determination are defined in Table 1.

In parallel, the samples were also determined by the reference method [28] aiming to evaluate the accuracy of the results obtained by these methodologies (Table 3). The application of the Student $t$-test for the pairs of values obtained illustrated the absence of statistical differences at a confidence level of 95% for the results obtained ($t = 0.157$ for the potentiometric/reference procedure; $t = 0.918$ for the fluorimetric/reference procedure). After evaluation of the relative errors corresponding to sample analysis, it is possible to conclude that the average of the results obtained by the two methodologies is more accurate since random errors are minimised.

4. Conclusions

This analytical system, in addition to operating automatically, is robust, rapid and enables the determination of diclofenac by two procedures based on two independent methodologies. The SIA system with accuracy assessment proved to be useful in the large scale quality control of diclofenac in pharmaceutical products, since it permits the evaluation of quality of results in real time and with an elevated sampling frequency.

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