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Sensitive sequential injection determination of naproxen based on interaction with β-cyclodextrin

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Abstract

A sensitive sequential injection analysis (SIA) methodology for the fluorimetric determination of naproxen is proposed. The developed automatic analytical procedure is based on the complexation of naproxen with β-cyclodextrin (β-CD) yielding an enhanced fluorimetric signal (λ_{ex} = 280 nm, λ_{em} = 356 nm).

Linear calibration plots were obtained for naproxen concentrations up to 1×10^{-5} mol l^{-1}. The developed methodology exhibited a good precision, with a R.S.D. < 2.1% (n = 15). The detection limit of the determination was 1.9×10^{-7} mol l^{-1} with a sampling rate of about 70 h^{-1}. The automatic method was applied to the determination of naproxen in pharmaceutical formulations. The obtained results were compared with those furnished by the reference procedure and the relative deviations were lower than 3.6%. No interference was found from the excipients usually used in solid pharmaceutical formulations.

Keywords: SIA; Fluorescence; β-Cyclodextrin; Naproxen

1. Introduction

Naproxen, (*S*)-6-methoxy-α-methyl-2-naphthaleneacetic acid (NAP), is a non-steroidal anti-inflammatory drug (NSAID), based on the aryl acetic structure (Fig. 1) that exhibits also analgesic and antipyretic properties. Its characteristics are thought to be related with the inhibition of cyclooxygenase, which prevents the biosynthesis of prostaglandin in human tissues and fluids. It is commonly used in the treatment of many diseases including rheumatoid arthritis, osteoarthritis, acute gout, primary dismenorrea and in the relief of mild to moderate pain [1].

NAP has been determined in pharmaceutical formulations using fluorimetric [2], voltammetric [3] and high performance liquid chromatographic [4] methods, all involving the use of organic solvents and tedious sample preparation and in the last one expensive equipment. Several sensitive batch phosphorimetric [5,6], isotachophoretic [7], chemiluminometric [8], and spectrophotometric [9] methods have been also used for the same purpose representing even though some limitations for routine analysis such as the constant dependence on the operator. Some studies using flow injection analysis methodologies based on direct UV [10] or fluorimetric [11,12] determination of NAP have also been reported. These methods present some drawbacks such as the use of organic solvents [11,12] and the laborious sample preparation [10,11].

The objective of the present work was then to develop a simple and sensitive method for the quantification of NAP in aqueous medium that could be an alternative to the already existing methods for the same purpose. The developed methodology was based on the enhancement of NAP native fluorescence (λ_{ex} = 280 nm, λ_{em} = 356 nm) in the presence of β-cyclodextrin (β-CD) Fig. 2.

Cyclodextrins are cyclic oligosaccharides constituted by six or more D-(+)-glucopyranose units that present an almost conical hydrophobic cavity being able to form inclusion com-

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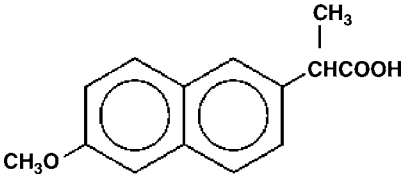

Fig. 1. Naproxen chemical structure.

plexes with a large variety of molecules with suitable polarity and dimension [13]. They have been used to increase aqueous solubility and chemical stability of drugs and to enhance drug delivery through biological membranes. Indeed the formation of an inclusion complex can change the physico–chemical properties of the guest molecule and besides the already referred situations, this fact can be useful from an analytical point of view [14]. The ability of CD to form inclusion complexes with better fluorescent properties than those of the free guest compound has been used for the determination of several substances in pharmaceutical formulations [15–18]. β-Cyclodextrin does usually possess better complexation efficiency with drugs than α- and δ-cyclodextrins and previous studies [19–21] showed that β-CD significantly modifies the fluorescence spectra of NAP due to a 1:1 complex formation. In order to accomplish the objective of this work, the determination of NAP, based on its complexation with β-CD, was performed automatically in a sequential injection analysis (SIA) system. This technique has already proved to be a robust and versatile sample handling approach that minimizes the consumption of sample and reagent solutions and the generation of effluents [22]. The computer controlled mode of operation of a SIA system, based on the forward and reversed flow of sample and reagent solutions through a multiposition valve, makes it a very reliable option in pharmaceutics routine analysis [23].

The association of the fluorimetric sensitive determination of NAP with the sequential injection technique intended to provide a fast, automatic and robust method suitable for routine quality control that could also be used for dissolution studies of solid formulations.

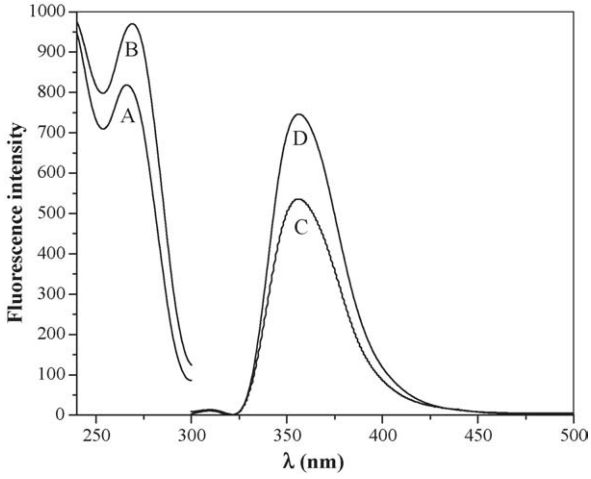

Fig. 2. Naproxen (5×10^{-5} mol l^{-1}) excitation (A and B) and emission (C and D) spectra in the absence (A and C) and in the presence (B and D) of β-CD (5×10^{-3} mol l^{-1}).

2. Experimental

2.1. Reagents

All solutions were prepared using chemicals of analytical reagent grade and high purity water (milli-Q), with a specific conductance <0.1 μS cm^{-1}, that was also used as carrier solution.

Stock solutions of naproxen 5×10^{-5} mol l^{-1} and β- and α-CD 5×10^{-3} mol l^{-1} were prepared by dissolving the appropriate amounts of powder in water. Standard solutions of naproxen were prepared daily from the stock solution by suitable dilutions.

The analysed pharmaceutical preparations were Naprosyn 250 and 500 mg (Roche), Naprosyn 500 mg granulated (Roche), Naprosyn 500 mg gastro resistant, Reuxen 250 and 500 mg (Tecnifar). Sample solutions of these pharmaceutical formulations were prepared by dissolving the required amounts of powdered tablets or granulated in water and then analysed directly in the flow system.

2.2. Apparatus

Fluorescence measurements were made on a Perkin-Elmer model LS 30 fluorescence detector equipped with a 7 μl flow-cell.

The SIA system (Fig. 3) consisted of a Gilson Minipuls 3, peristaltic pump, equipped with a PVC pumping tube (1.2 mm i.d.) and a 10-port multiposition Vici Valco selection valve. Manifold components were connected by means of PTFE tubing, 0.8 mm i.d., which was also used for the holding and reaction coil (4 and 0.5 m, respectively). A solenoid valve (SV, Fig. 3) and a contact device (on the peristaltic pump), similar to the one described before by Araújo et al. [24], controlled the pump starting point in order to guarantee reproducibility in the solution aspirated or propelled volumes.

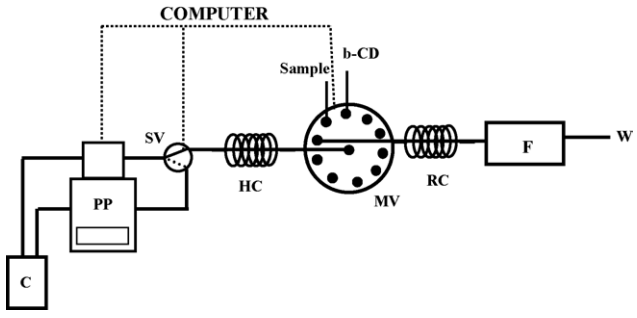

Fig. 3. Sequential injection analysis system used for the determination of naproxen. C—carrier (water); PP—peristaltic pump; SV—solenoid valve; MV—multiposition valve; HC—holding coil (4 m, straight); RC—reaction coil (0.5 m, figure eight); F—fluorimetric detector; W—waste.

Analytical system control, including the operation of the peristaltic pump, selection valve and solenoid valve, was achieved by means of an Advantech PCL 711B interface card and a Pentium-I based microcomputer. Software was developed in Microsoft Quick-Basic and permitted to control flow rate, flow direction, valve position, sample and reagent volume and data acquisition and processing. During optimisation, the analytical signals were also recorded on a Kipp & Zonen BD 111 strip chart recorder.

Sample analysis according to the reference method recommended by the US Pharmacopoeia (USP 24) [25], was carried out in a Merck Hitachi Lachrom Liquid Chromatograph, equipped with an L-7455 Diode Array detector, an L-7100 pump and a D-7000 interface card.

2.3. Reference procedure

The assay of the reference method [25] was performed using a mixture of acetonitrile, water and glacial acetic acid (50:49:1) as mobile phase and a mixture of acetonitrile and water (90:10) as solvent mixture. Butyrophenone was used as internal standard. The tablets were weighed, powdered and the equivalent to 250 mg of NAP was transferred to a 100 ml flask. Ten millilitres of water were added to the powder and the mixture was sonicated for 10 min. Then 80 ml of acetonitrile were added to the flask and the mixture was sonicated for 5 min. The volume was then completed with acetonitrile and 1 ml of the resulting solution was mixed with the internal standard and diluted to 100 ml with mobile phase.

3. Results and discussion

The proposed methodology was developed in two stages in order to establish first the initial conditions of the reaction and second the final conditions of the assay. In this way, before the optimisation of the automatic procedure, some batch studies were performed in order to evaluate NAP specrofluorimetric properties, in aqueous medium, in the presence and in the absence of β-CD. These studies showed that not only NAP exhibits native fluorescence at 356 nm when excited at 280 nm, but also that there is an enhanced fluorescence signal in the presence of β-CD at the same excitation and emission wavelengths.

3.1. Optimisation of the physical and chemical parameters of the SIA methodology

After the establishment of the initial conditions of the reaction, the next objective was the implementation of the determination of NAP with β-CD in a sequential injection analysis system. The optimisation studies involved not only the study of the physical and chemical parameters of the automatic system but also a parallel study of NAP native fluorescence behaviour. The optimisation of each parameter in the automatic system included the evaluation of NAP analytical signals in the presence and in the absence of β-CD, so that two calibration plots were obtained. These studies meant to evaluate the increment of NAP fluorescence signal in the presence of β-CD and also the change in the most important figures of merit of the determination, related with its use.

Regarding the optimisation of the analytical procedure itself, several studies were performed with the aim of investigating the influence of sample and β-CD volume, β-CD concentration, order of aspiration, flow rate, reaction coil length and configuration and pH of the carrier solution in the formation of an inclusion complex between NAP and β-CD and consequently on the respective fluorescence signal.

The influence of NAP solution volume on the fluorescence signal was tested between 25 and 100 μl being all the following studies performed using 50 μl of sample. Although it was verified that the analytical signal increased with increasing sample volumes until 100 μl (Fig. 4, sv), above 50 μl there was detector's saturation for standards with concentration higher than 5×10^{-6} mol l^{-1}.

Due to its importance to the complex formation, β-CD volume was studied in the range of 25–100 μl. The optimisation proceeded with 50 μl since higher volumes did not lead to a significant increase on the signal and tended to cause dilution of the formed product (Fig. 4, cdv). Regarding β-CD concentration it was concluded that the fluorescence intensity increased with increasing β-CD concentrations up 5×10^{-3} mol l^{-1}, above which it remained constant (Fig. 4, cdc). Solutions with concentrations above 7.5×10^{-3} mol l^{-1} exhibited solubility problems. Details about the general effect of β-CD on the determination of NAP will be discussed later on this paper (Analysis of the pharmaceutical formulations).

Similar studies were performed in the presence of α-CD but no variation on the native fluorescence of NAP was observed.

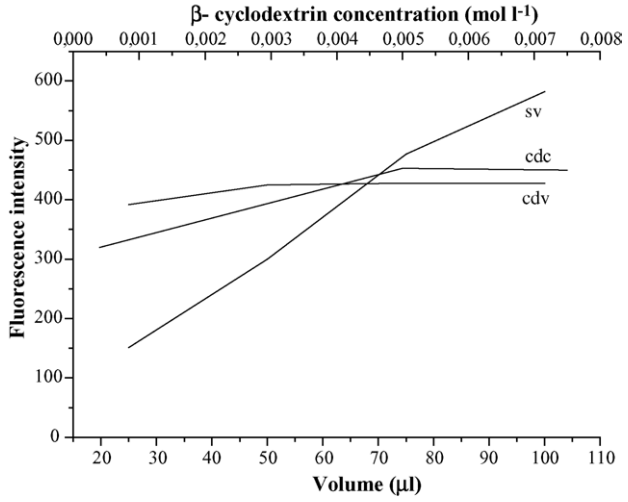

Fig. 4. Effect of sample volume (sv) and β-CD volume (cdv) and concentration (cdc) on the fluorescence signals of a 5×10^{-6} mol l^{-1} naproxen standard.

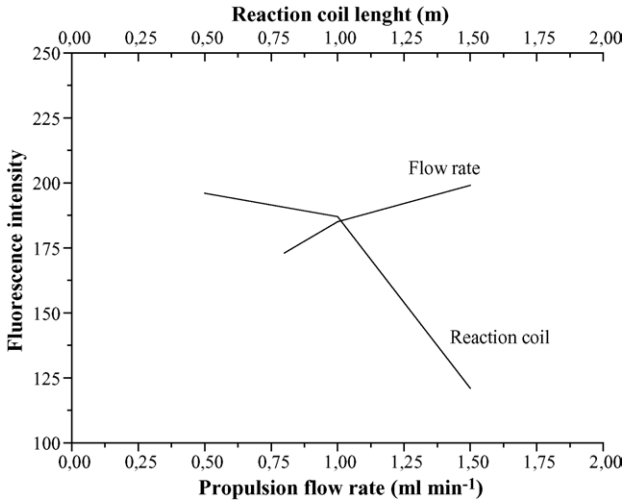

Fig. 5. Effect of reaction coil length and flow rate on the fluorescence signals of a 5×10^{-6} mol l^{-1} naproxen standard.

In a SIA system the sequence of aspiration of sample and reagents into the holding coil, determines how the different zones will mutually inter-disperse and the degree of dispersion. As a result of a lower dispersion of the sample zone and a higher reagent zone penetration, an increase in the analytical signals of about 5% was observed when β-CD and sample zones were sequentially aspirated to the holding coil, showing a better mixing efficiency in this situation.

The influence of propulsion flow rate was evaluated in order to establish the optimum residence time of the reaction zone inside the flow system allowing an adequate reaction development and avoiding dilution of the formed complex. The results obtained during the study of the flow rate between 0.8 and 1.5 ml min^{-1} showed that the reaction was relatively fast. There was an increase of about 1.15 times in the analytical signals, until 1.5 ml min^{-1}, revealing that the residence time was excessive for lower flow rates (Fig. 5, flow rate). However, the remaining studies were performed propelling the reaction zone to the detector at 1 ml min^{-1} since the utilization of higher flow rates resulted in a saturation of the detector with the more concentrated standards (1×10^{-5} mol l^{-1}).

The better residence time was evaluated by determining the effect of the reaction coil length and configuration on the analytical signals. Reaction coils above 0.5 m resulted in a decrease of about 60% of the analytical signals as a result of an excessive dilution of the formed complex. Of the different tested configurations (straight, coiled, figure eight) figure eight reactors led to higher analytical signals in the studied concentration range confirming the fact that they originate lower dispersion of the reaction zone on its way to the fluorescence detector. For all this the optimisation proceeded with a 0.5 m figure eight reaction coil (Fig. 5, reaction coil).

The ionisation of NAP, determined by the pH of the reaction medium, is theoretically very important for the complex formation extension with β-CD. It is effectively known that CD cavity has preferential affinity for the unionised forms of weak carboxylic acids [13]. A study of the influence of the pH of the carrier solution on the analytical signals was then performed using a solution of sodium hydrogen carbonate 0.06 mol l^{-1} and boric acid 0.1 mol l^{-1} for pH between 7 and 10, 1 mol l^{-1} acetic acid for pH 4 and 0.2 mol l^{-1} hydrochloric acid for pH 2. The obtained results did not show a significant variation of the analytical signals with the change of the carrier pH. As the reaction zone only takes about 20 s to get to the fluorimetric detector, maybe there is not enough time to cause significant changes in the ionisation of NAP. As no variation on the analytical signals was observed, the remaining studies were performed as before using water as carrier.

Possible interference of compounds such as sacharose, glucose, fructose and lactose, which are commonly used as excipients in solid pharmaceutical formulations, was studied. A specie was considered as non-interfering when the analytical signal variation regarding the one obtained in its absence was lower than 3%. Standard solutions of NAP 5×10^{-6} mol l^{-1} and increasing concentrations of the excipients were analysed by the SIA methodology and it was observed that up to a 50 molar ratio NAP/lactose and up to a 100 molar ratio NAP/other excipients (highest value tested) no interfering effect was noticeable.

3.2. Figures of merit

After the optimisation of all the parameters affecting the β-CD–NAP complex formation, the developed methodology was evaluated for NAP concentrations up to 1×10^{-5} mol l^{-1} and linear calibration plots were obtained. The typical calibration plots obtained in the presence and in the absence of β-CD were $IF = 8 \times 10^7(\pm 2 \times 10^6)C + 4.8(\pm 12.0)$ ($n = 7$; $R^2 = 0.9994$) and $IF = 6 \times 10^7(\pm 2 \times 10^6)C + 1.1(\pm 10.7)$ ($n = 7$; $R^2 = 0.9993$), respectively (IF—fluorescence intensity; C—NAP concentration, mol l^{-1}). The detection limits [26] of the determination of NAP with and without β-CD were 1.9×10^{-7} and 1.1×10^{-6} mol l^{-1}, respectively. The sampling rate was about 70 samples/h.

3.3. Analysis of the pharmaceutical formulations

The developed methodology, based on the enhanced fluorescence signal of NAP in the presence of β-CD, was applied to the determination of NAP in pharmaceutical formulations. In order to evaluate the accuracy of the proposed SIA methodology the obtained results were compared with those furnished by the HPLC reference procedure. No significant differences were observed between the results obtained by both methods with a relative deviation lower than 3.6% (Table 1).

A linear relationship between the two methods was established: SIA (mg) = 0.967(±0.045) Ref (mg) + 6.29(±19.96), along with the evaluation of the correlation significance using the t-test, carried out as a bilateral coupled test, according

Table 1
Results of the analysis of the pharmaceutical formulation by the developed SIA procedure and the HPLC reference procedure

	SIA ± S.D.[a] (mg/formulation)	HPLC ± S.D.[a] (mg/formulation)	Error[b] (%)
Naprosyn 250 mg	242 ± 0	246 ± 1	−1.6
Naprosyn 500 mg	494 ± 7	501 ± 2	−1.4
Naprosyn 500 mg gastro resistant	530 ± 3	537 ± 2	−1.4
Naprosyn 500 mg granulated	500 ± 1	510 ± 1	−2.0
Reuxen 250 mg	251 ± 4	250 ± 2	+0.4
Reuxen 500 mg	487 ± 2	505 ± 4	−3.6

[a] S.D.: standard deviation of four (4) replicates.
[b] Error = [(mg of naproxen by SIA − mg of naproxen by HPLC)/mg of naproxen by HPLC] × 100.

to Miller [26], using $t = (\bar{X}/S)\sqrt{n}$ with $n-1$ degrees of freedom ($\bar{X}$ is the mean difference between each pair, S the standard deviation and n is the number of measurements). The calculated t-value (1.61), when compared with the tabulated value at the 95% confidence level (2.78), confirms the good agreement between the two methods, since the null hypothesis is accepted.

No significant differences (R.S.D. < 2.1%) were obtained in the repetitive analysis ($n = 15$) of samples with different concentrations of NAP (9×10^{-5} and 4.9×10^{-5} mol l^{-1}) confirming the repeatability of the developed procedure.

4. Conclusions

Regarding the already existing methods for the determination of NAP in pharmaceutical formulations [2–12] the automatic method presents several advantages resulting from the combination of the sequential injection analysis technique with the sensitivity of the fluorimetric determination of the complex formed between NAP and β-CD. Effectively, the developed procedure showed to be robust, simple and fast leading to a very low reagent and sample consumption and effluent production. In each determination, 0.28 mg of β-CD were spent and 1.1 ml of effluent was generated. Besides this, it is possible to carry out the determination without any previous separation, extraction or other tedious procedures regarding sample preparation. When compared with the referred flow methods [10–12] used for the same purpose the developed methodology showed to be more independent from the operator, sensitive [10,11] and simple avoiding the utilization of organic solvents.

Moreover, the utilization of β-CD showed to be, as reported by other authors, a good host to promote fluorescence of NAP in aqueous solution. This fact resulted in a lower detection limit allowing the application of the methodology to the determination of NAP in other kinds of sample like human serum or urine in which the concentrations are very low.

The automatic methodology for the determination of NAP in pharmaceutical formulations, based on the complexation of NAP with β-CD, for all the exposed advantages is suitable for routine quality control and can be seen as an alternative to the already existing methods.

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