BIOMEDICAL CHROMATOGRAPHY
Biomed. Chromatogr. **13**: 145–147 (1999)

Optimization of intrinsic naproxen fluorescence by off-line and flow injection analysis

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Received 27 May 1998; accepted 18 June 1998

INTRODUCTION

Naproxen, ((d)-2-(6-methoxy-2-naphthyl)-propionic acid) belongs to the propionic class of non-steroidal anti-inflammatory drugs (NSAIDs). It is an important agent with anti-inflammatory, analgesic and antipyretic properties and has proved to be effective in the symptomatic treatment of rheumatoid arthritis and adult osteoarthritis. It is well absorbed in the upper gastrointestinal tract when administered orally and is almost completely bound to plasma proteins.

The technique of flow injection analysis (FIA) is a most useful tool for the rapid and automated analysis of drugs (Calatayud, 1996; Palilis *et al.*, 1997). Small volumes of samples and reagents are used thus minimizing waste disposal. Fluorometric methods of detection (Mahedero and Aaron, 1992; Chen *et al.*, 1991; Baeyens *et al.*, 1997) provide excellent sensitivity and detection often exceeds limits reached by spectrophotometric methods. In addition, fluorometry offers the advantage of less interference by compounds present in the matrix when compared to UV absorbing techniques.

The purpose of the present study was to develop a simple and sensitive FIA method for the quantitation of naproxen in pharmaceutical preparations. Therefore, different factors influencing the intrinsic fluorescence properties of naproxen were investigated.

EXPERIMENTAL

The sodium salt of naproxen was purchased from Sigma-Aldrich (Bornem, Belgium). Deionized water was used throughout. All other chemicals and solvents used were of analytical grade and used as such without extra purification. The pH values of the solutions were controlled with a Metrohm 691 pH meter (Pleuger, Belgium) with a combined Ag/AgCl-electrode.

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Fluorescence spectra (uncorrected) and measurements were made with a fluorescence spectrophotometer Shimadzu RF-5001 PC (Shimadzu, Kyoto, Japan) equipped with a 150 W Xenon lamp. The fluorimeter was operated at high sensivity settings, low scanning speed and excitation and emission slits of 3.0 nm.

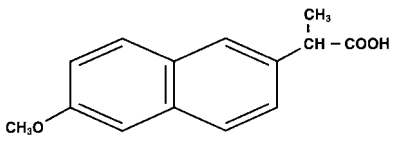
The FIA configuration was built up by the following instruments: pump, Shimadzu LC-9A liquid chromatograph (Shimadzu, Kyoto, Japan); injector, Valco type U six-port (Valco Instruments Co., Houston, TX). Detection was performed with a Shimadzu Model RF-551 fluorescence HPLC monitor (Shimadzu, Kyoto, Japan) equipped with a 2 mL quartz flow-cell. Integration was carried out with a Shimadzu Chromatopac C-R6A recording integrator.

RESULTS

Influence of pH and type of buffer

From a stock solution of sodium naproxen ($0.01\,mg\,mL^{-1}$) 1.0 mL was diluted with 9.0 mL of different buffer solutions (citrate, phosphate and borate buffers) prepared in the 1–13 pH range. With organic acid buffers (citrate, phthalate) and applying an excitation wavelength of 233 nm (emission measured at 353 nm) low fluorescence emission was noted when increasing pH values from 1 to 5. When using excitation wavelengths of 272 nm or 328 nm, no changes in fluorescence intensity over the entire pH range occurred although signals were lower than when exciting at 353 nm (Fig. 1).

Mineral buffers (phosphate 0.05 M, pH 3; borate 0.05 M, pH 9) proved the best choice for high fluorescence intensities applying excitation and emission monochromator wavelength installations at 232 nm and 353 nm,



Structure 1. Naproxen

CCC 0269–3879/99/020145–03 $17.50

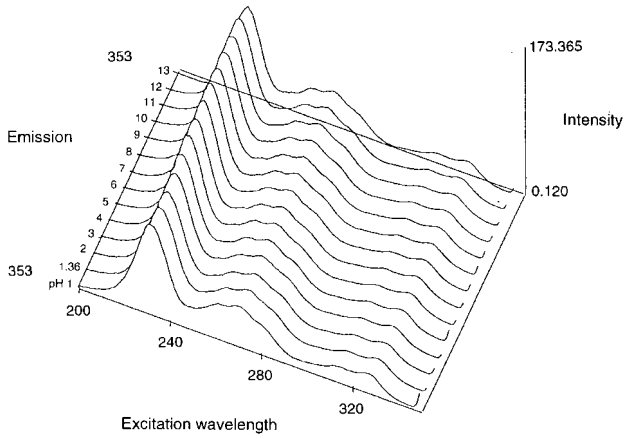


Figure 1. Excitation spectra of sodium naproxen in function of pH (pH 1–8: phosphate buffer, 0.05 M; pH 9–13, borate buffer, 0.05 M). Emission monochromator at 353 nm.

respectively. When exciting at 272 nm or 328 nm, lower relative fluorescence intensities were noticed, depending less, however, on the type of buffer.

Influence of enhancers

With monochromator settings of 233 nm and 353 nm, for excitation and emission, respectively, an increase of 25%, 40% and 50% of relative fluorescence emission was noticed when adding, respectively, 50 mM sodium dodecyl sulphate, 10 mM β-cyclodextrin hydrate and 10 mM Brij 35 to the three different buffers (phosphate 0.05 M, pH 3; phosphate 0.05 M, pH 7 and borate 0.05 M, pH 9). Because of the substantial fluorescence enhancing effect by Brij 35, this compound was further investigated in a concentration range from 0–100 mM (Fig. 2). A concentration of 30 mM proved an optimum value. Commonly used additives in pharmaceutical preparations (cellulose, magnesium stearate, polyvidone, talc) did not cause any influence on the observed fluorescence intensities, as expected.

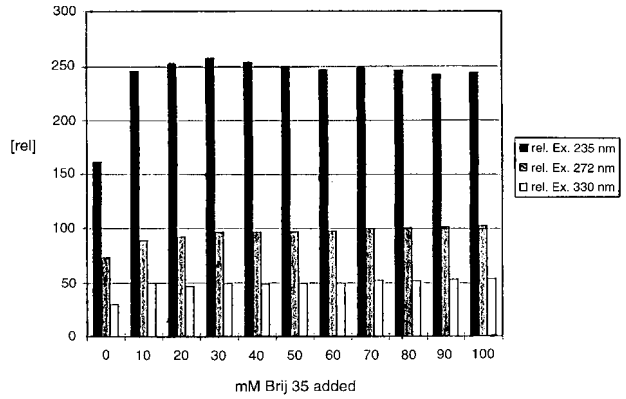


Figure 2. Influence upon fluorescence intensity of Brij 35 added to a solution of sodium naproxen in 0.05 M phosphate buffer, pH 3. Emission monochromator at 353 nm.

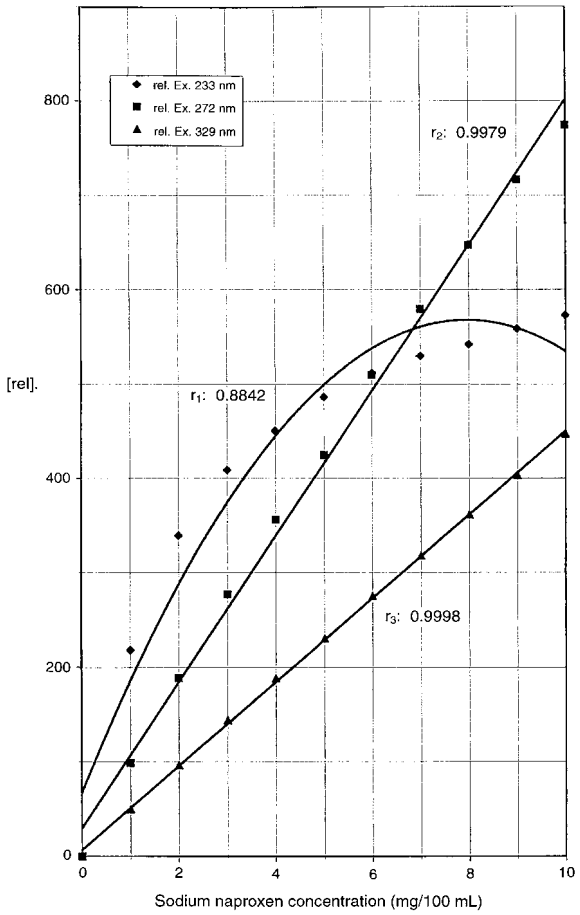


Figure 3. Calibration curve of sodium naproxen (0.01–0.1 mg mL^{-1}) in 0.05 M phosphate buffer, pH 3 in the presence of 30 mM Brij 35 at different excitation wavelengths. Emission monochromator at 353 nm.

Influence of organic solvents

Organic solvents (methanol, acetonitrile, 1-propanol, 2-propanol and tetrahydrofuran), frequently used in RP-HPLC set-ups and required in the present study for dissolving pure naproxen acid, were investigated with respect to their effects upon fluorescence properties of the analyte. In all cases an increase of fluorescence intensity was noticeable (about 10% when adding 10% (v/v) solvent) up to a concentration of 30% (v/v) organic modifier, followed by a slight decrease using a 50% (v/v) ratio, except for methanol where a slower increase was observed without subsequent fluorescence decrease.

Linearity

Linearity was controlled in the concentration range from 1–10 mg/100 mL, in phosphate buffer 0.05 M, pH 3. Employing an emission monochromator installed at 353 nm, excellent linearity was observed in all cases when exciting at 329 nm (Fig. 3). When exciting at 233 nm the following *r*-values were obtained: 0.92, 0.88

and 0.72, without enhancer, with Brij 35 and using 20% acetonitrile, respectively.

Off-line determination

Sodium naproxen was determined in a pharmaceutical preparation (tablets 275 mg sodium naproxen) after extraction with water containing 20% (v/v) acetonitrile and 30 mM Brij 35 for 15 minutes in an ultrasonic bath. Further dilutions were made with the same solvent including phosphate buffer 0.05 M, pH 3. With a calibration range of 2–10 mg/100 mL ($n = 6$, $r = 0.9992$) the determination resulted in a drug content value of 281.8 mg ($n = 10$, rsd 1.08), i.e., 102.5% with respect to the label value.

On-line determination

For the FIA set-up, phosphate buffer 0.05 M, pH 3 containing 30 mM Brij and 20% (v/v) acetonitrile was used as the mobile phase. On-line spectra and influence of flow-rate and of detector response factor were investigated. Using the same calibration range as above ($n = 3$, $r = 0.9999$) the determination resulted in a value of 288.8 mg ($n = 6$, rsd 0.17), i.e. 105.0% with respect to the label value.

CONCLUSION

FIA, or continous-flow analysis, definitely has become a mature technique. After optimizing chemical parameters in order to provide a suitable carrier (pH, type of buffer, enhancers, solvents), and detection parameters (monochromator wavelength installations), three factors influencing the intrinsic fluorescence of naproxen were optimized, followed by optimization of typical FIA parameters (flow-rate, response factor, connections and injection volume). Optimization in a FIA set-up allows the influence of a number of experimental variables, with critical effects upon the produced signal, to be rapidly evaluated and contributes to extrapolations in terms of HPLC separations. It is clear that mixtures of compounds or decomposed samples should be quantified employing chromatographic or capillary electrophotoretic separating techniques.

Compared with conventional, non-separational methods for the quantification of naproxen in pharmaceutical preparations, FIA offers an elegant technique, as well as being attractive from the point of view of reagent consumption and time of analysis, without any loss of precision. Although no separation process is involved, a high number of analyses can be performed in a restricted time period, which is most convenient especially in the case of bulk product analysis.

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