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Diclofenac and Naproxen Analysis by Microbore Liquid Chromatography (LC) with Native Fluorescence Detection

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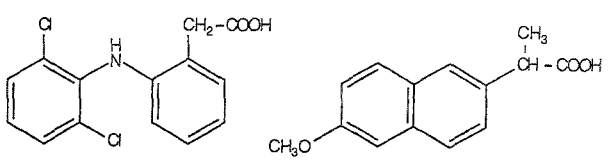
A microbore LC system applying native fluorescence detection is described for analysis of the nonsteroidal antiinflammatory agents diclofenac and naproxen in pharmaceutical preparations, allowing the visualization of some diclofenac decomposition products. The suggested system is practical and specific and offers detection limits of 0.2 μg ml^{-1} for diclofenac and of 1 pg ml^{-1} for naproxen (solutions to be injected). Moreover, the solvent consumption is reduced by a 10-fold factor compared to the classical HPLC setup.

KEY WORDS: Microbore liquid chromatography; fluorescence detection; diclofenac; naproxen; nonsteroidal antiinflammatory agents.

INTRODUCTION

On the occasion of the general trend to miniaturize analytical liquid chromatographic separating systems in order to reduce organic waste and reagent cost of analysis, a microbore reversed-phase liquid chromatographic system employing native fluorescence detection was developed to determine the drugs diclofenac and naproxen in pharmaceutical formulations, as well as to contribute to the general drug stability data obtained via classical RP-HPLC with UV detection. Diclofenac ([2-(2,6-dichloroanilino)phenyl]acetic acid) **(I)** [1,2] and naproxen [2-(6-methoxy-2-naphthyl)propionic acid] **(II)** [3,4] are widely used nonsteroidal antiinflammatory agents.

As miniaturization in liquid chromatography [5–12] often brings along poorer limits of detection, due to the generally reduced amounts of analyte injected and to the decreased volume of detector flow cell, often creating the need for postcolumn derivatization systems, the more sensitive and specific detection systems based on fluorescence measurements were considered to circumvent this drawback, adding extra dimensions to the results provided.



I II

Scheme I

Stimulated by the promising results obtained by this research group on the occasion of microscale packed-capillary liquid chromatography for the determination of biomedically important thiols after fluorigenic labeling [13–15], and comparison of the technique with capillary electrophoresis [16], the present investigation applies fluorescence detection of the intrinsic fluorescence properties of both drugs separated on a reversed-phase microbore (1-mm-i.d.) system, employing wavelength programmation of the installed excitation and emission pairs.

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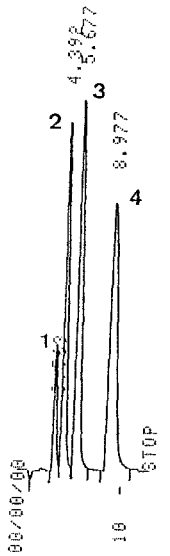

Fig. 1. Microbore liquid chromatographic separation of diclofenac and naproxen from diclofenac decomposition products. Conditions: column, Spherisorb (RP18) S5 ODS2 (15 cm × 1 mm, 5-μm particles); injection volume, 2.5 μl (homemade loop); mobile phase, acetic acid (2 ml L^{-1} to pH 4 using NaOH solution) + CH_3CN, 600 + 400; flow, 100 μl min^{-1}; temperature, 35°C; detection, fluorescence, flow cell, 2 μl; wavelengths, $\lambda_{exc.}$ = 292 nm, $\lambda_{em.}$ = 355 nm. Peaks 1 and 3, diclofenac degradations; 2, Na-naproxen; 4, Na-diclofenac.

EXPERIMENTAL

Chemicals

Acetonitrile for the mobile phase was of analytical grade (LabScan, Dublin, Ireland). Sodium diclofenac and naproxen were purchased from Sigma-Aldrich (Bornem, Belgium). Glass-distilled deionized water was used throughout. All other chemicals and solvents used in this study were of analytical grade (Merck, Darmstadt, Germany, and Janssen Chimica, Geel, Belgium) and used as such without extra purification.

Instruments

Isocratic chromatography was performed using the following instruments: pump, Shimadzu LC-9A liquid chromatograph (Shimadzu, Kyoto, Japan); injector, Valco Type U six-port (Valco Instruments Co., Houston, TX). Microbore Spherisorb S5 ODS2 columns (RP18, 15 cm × 1 mm, 5 μm) were purchased from Phase Separations (Deeside Clwyd, UK). Detection was performed with a Shimadzu Model RF-551 fluorescence HPLC monitor (Shimadzu, Kyoto, Japan), equipped with a 2-μl quartz flow-cell. Integration was carried out with a Shimadzu Chromatopac C-R6A recording integrator (Shimadzu, Kyoto, Japan). For comparative purposes, a Pye Unicam Model PU 4025 UV Detector containing an 8-μl flow-cell and a Waters Model 470 scanning fluorescence detector (Millipore Corp., Milford, MA) with 16-μl flow-cell were linked in separate experiments to a standard cartridge RP column (Lichrocart 125-4 Lichrospher RP18, 5 μm (Merck, Darmstadt, Germany).

Chromatographic Conditions

Solutions of sodium diclofenac and of naproxen in aqueous acetonitrile were injected.

Column:	Spherisorb (RP18) S5 ODS2 (15 cm × 1 mm 5 μm particles).
Injection volume:	2.5 μl (homemade loop).
Mobile phase:	acetic acid (2 ml L^{-1} to pH 4 using NaOH solution) + CH_3CN, 600 + 400.
Flow:	100 μl min^{-1}.
Temperature:	35°C.
Detection:	Fluorescence flow-cell, 2 μl; wavelength maxima for
	Diclofenac: $\lambda_{exc.}$ = 292 nm; $\lambda_{em.}$ = 355 nm
	Naproxen: $\lambda_{exc.}$ = 239 nm; $\lambda_{em.}$ = 351 nm

RESULTS AND DISCUSSION

Diclofenac quantitations could be carried out using naproxen as the internal standard, and vice versa, based on the separation of both drugs in the system described and, obviously, because of the separation obtained between the diclofenac decomposition products from both analytes, as illustrated in Fig. 1. For the analyses of the pharmaceutical preparations, the wavelength values were automatically switched after about 7 min (with autozeroing) between naproxen ($\lambda_{exc.}$ = 239 nm; $\lambda_{em.}$ = 351 nm) and diclofenac ($\lambda_{exc.}$ = 292 nm; $\lambda_{em.}$ = 355 nm).

Sodium Diclofenac

Linearity was controlled in the range 10–500 μg ml^{-1} (injected solution). The following results were obtained from the calibration curve: correlation coefficient, 0.999; limit of detection, 0.2 μg ml^{-1} (S/N=3; 2.5 μl injected volume); limit of quantitation, about 2 μg ml^{-1}.

The native fluorescence detection system, in spite of the poorer quantification limits in the microbore setup (about 40 times lower) compared to classical RP-LC with UV detection (C18, 4 mm × 12.5 cm, 5 μm),

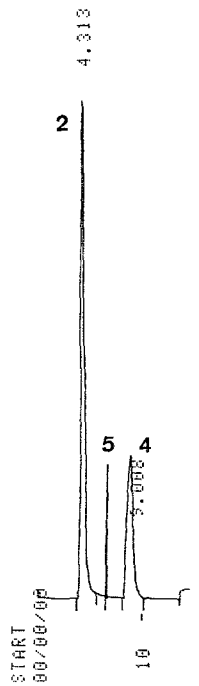

Fig. 2. Typical chromatogram obtained from the analysis of naproxen-containing pharmaceutical preparations. Conditions: see Fig. 1, with wavelength switching from $\lambda_{exc.}$ = 239 nm, $\lambda_{em.}$ = 351 nm, to $\lambda_{exc.}$ = 292 nm, $\lambda_{em.}$ = 355 nm. Peak 2, Na-naproxen; peak 4, Na-diclofenac; peak 5, wavelength-pair-switching signal.

proved more valuable for quality control purposes of diclofenac. As shown in Fig. 1, the fluorescence-detected chromatogram clearly indicates two extra peaks—not noticeable in UV-detected systems—exhibiting increasing areas in function of time, indicating the relatively high yields of fluorescence of these decomposition products. Applying the UV-detection mode, no extra peaks could be noticed after 24 h standing of aqueous acetonitrile solutions of diclofenac, providing only negligible loss of content after quantitation. Hence, fluorescence-based detection is to be preferred for quality-control purposes of diclofenac.

Repeatability tests for the injection of an aqueous reference solution (0.2 mg ml^{-1} of Na-diclofenac) provided an RSD of 1.81% (n=10) on the 1-mm column.

Naproxen

Linearity was controlled in the range 1–100 ng ml^{-1} (injected solution). The following results were obtained from the calibration curve: correlation coefficient, 0.998; limit of detection, 1 pg ml^{-1} (S/N=3; 2.5 µl injected volume; highest sensitivity settings of detector).

Due to the baseline noise and because of the high native fluorescence properties of naproxen, the instrument was installed at low sensitivity settings for the first 7 min of the chromatographic elution.

Repeatability tests for the injection of a reference solution (2 µg ml^{-1} of naproxen in aqueous acetonitrile) provided an RSD of 0.51% (n=10) on the 1-mm column and 0.62% on the 4-mm column.

Analysis of Pharmaceutical Preparations

For the analysis of pharmaceutical formulations containing Na-diclofenac and naproxen via their mutual use as internal standards, solutions of Na-diclofenac and of naproxen were prepared by dissolving the compounds in 20% aqueous acetonitrile followed by dilution in the same solvent; the final solutions for injection being prepared by dilutions in pure water. Figure 2 illustrates a typical chromatogram obtained from the analysis of pharmaceutical preparations.

For analysis of the solution for injection containing Na-diclofenac, a concentration range of 0.1–0.3 mg ml^{-1} was chosen and the concentration of the internal standard (naproxen) was about 0.04 µg ml^{-1}.

The determination of diclofenac in a pharmaceutical preparation (solution for injection), label content 25.0 mg ml^{-1}, of Na-diclofenac yielded an average content of 24.7 mg ml^{-1} (n=8), with an RSD = 0.6%.

In the case of the tablets containing naproxen the concentration range was chosen between 1 and 3 µg ml^{-1} (low sensitivity setting) with a final internal standard (diclofenac) concentration of 0.3 mg ml^{-1}.

The determination of naproxen in a pharmaceutical preparation (tablets, label content of 275 mg Na-naproxen) yielded an average content of 273.9 mg per tablet (n=3), RSD = 1.39%. The same naproxen solutions were analyzed on the 4-mm column applying 20-µl injections, using the Waters Model 470 fluorescence detector, similarly applying gain switching and wavelength programming in excitation and in emission modes in function of time, as described above. An average content of 276.1 mg per tablet (n=3) was obtained, RSD = 1.09%. Tablets analysis substituting fluorescence detection by UV (277 nm) detection yielded an average content of 273.4 mg per tablet.

CONCLUSION

In spite of the poorer quantification limits for diclofenac the detection system based on fluorescence proved more valuable for quality-control purposes as the

formation of decomposition products could be adequately followed, which was not possible in the classical mode using UV detection.

The application of the intrinsic fluorescence properties of diclofenac and of naproxen in the microbore setup offers extra specificity features to the quality-control requirements of modern analytical methods. Moreover, detection of native naproxen fluorescence, based on its strongly fluorescence emitting methoxynaphthalene nucleus, proves to be most sensitive. Furthermore, the acetonitrile consumption of the described microbore system is reduced by a 10-fold factor. Finally, it should be stated that the use of microbore columns is not limited to specific microcell-equipped fluorescence detectors. Larger cells (>>2 μl) may likewise be used, leading to an improvement of detection limits, however, at the expense of resolution loss. Further work on this topic is in progress by the present research group.

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