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Note

Rapid high-performance liquid chromatographic method for the quantitative determination of diflunisal in plasma

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Diflunisal [5-(2′4′-difluorophenyl)salicylic acid] is a new ring-substituted salicylic acid derivative with promising analgesic and anti-inflammatory properties which make the drug useful for the treatment of osteoarthritis [1, 2]. Recent studies [3—5] indicate that the drug exhibits a dose-dependent pharmacokinetic profile. As compared to other salicylates diflunisal has a long plasma half-life time which, similarly to salicylic acid itself [6], increases with increasing dose. This rather long $t_{1/2}$ is especially relevant since it permits a dosage regimen with only one or two daily doses. On the other hand the capacity limited elimination rate implicates the necessity of monitoring plasma drug levels for the establishment of optimal dosage regimens.

For the assay of drug levels in the course of a clinical pharmacological study comparing the therapeutic efficacies of diflunisal and of naproxen, a rapid and specific assay method for these drugs was needed.

In this report a description of a high-performance liquid chromatographic (HPLC) method for the determination of diflunisal is given, which combines a short-time analysis with ease of handling. The procedure is based upon almost quantitative extraction of the diflunisal from plasma followed by reversed-phase liquid chromatography with UV spectrophotometric detection. As an internal standard the analgesic naproxen (6-methoxy-α-methyl-2-naphthaleneacetic acid) is used. This method has several advantages in comparison with published procedures involving spectrofluorometry [4] and has been already applied in preliminary pharmacokinetic studies.

MATERIALS AND METHODS

Materials

Diflunisal, a pure substance for reference purposes, was kindly provided by Merck, Sharp and Dohme (Brussels, Belgium) and naproxen by Syntex (Palo Alto, CA, U.S.A.).

The column-filling material, LiChrosorb RP-8 (5 μm) was obtained from Merck (Darmstadt, G.F.R.) and the tetramethylammonium hydrogen sulphate, used as a counter-ion, from Fluka (Buchs, Switzerland). All other reagents were analytical grade products from Merck. The diethyl ether was distilled shortly before use.

Stock solutions of diflunisal and naproxen were prepared by dissolving 25 mg of either drug in 100 ml of methanol.

Apparatus

A Hewlett-Packard 1084B high-pressure liquid chromatograph equipped with an autosampler and a HP 79850 LC terminal was used (Hewlett-Packard, Waldbronn, G.F.R.). The column (stainless steel, 15 cm × 0.46 cm I.D.) was packed with LiChrosorb RP-8 (5 μm) and eluted with a methanol—water mixture (50:50), containing 0.01 *M* tetramethylammonium hydrogen sulphate and Tris [tris(hydroxymethyl)aminomethane]. The apparatus was operated at a column temperature of 32°C and a flow-rate of 1.4 ml/min. The column pressure was $26 \cdot 10^3$ KPa. The eluting compounds were detected and measured at 254 nm.

Extraction

Plasma (0.5 ml) containing diflunisal, is pipetted into a test tube provided with a screw-cap. Naproxen (50 μg) in methanolic solution (0.2 ml) is added. After thorough mixing on a whirlmixer 5 ml of a diethyl ether—*n*-hexane (50:50) mixture and 0.7 ml of a 1.5 *N* hydrochloric acid solution are added. The closed tube is mechanically shaken for 30 min and then centrifuged for 15 min at 1500 *g*. The organic layer is transferred into another test tube and evaporated to dryness at 30°C under a gentle stream of dry, filtered air. The residue is taken up into 1 ml of methanol of which 10 μl are injected into the liquid chromatograph.

Calibration and recovery

The procedure is calibrated by programming the LC terminal in the internal standard. Various known amounts of diflunisal and a fixed amount of naproxen (50 mg/l) are added to blank plasma. These samples are analysed as outlined above and calibration graphs are obtained by plotting the concentration of diflunisal calculated by the terminal (from integration of the signal) against the concentration added, assuming a linear relation between the surfaces under the curves of the different diflunisal concentrations and the 100 mg/l concentration as a reference.

The overall recovery of the procedure is determined by comparison of the values obtained after extraction with values obtained after direct injection of standard solutions.

RESULTS AND DISCUSSION

Under the conditions employed no interferences by endogenous plasma constituents occurred in the chromatogram. For example, Fig. 1 shows a chromatogram obtained from blank plasma as well as one from plasma containing diflunisal and naproxen. A whole run is completed within 10 min, the retention times of naproxen and diflunisal being about 2.5 and 4.2 min respectively.

Linear calibration curves were obtained over the concentration range of 5—100 mg/l of diflunisal in plasma. The limit of detection of diflunisal with the method described is substantially below 5 mg/l, but in order to obtain reproducible, quantitative results at such low concentrations a separate calibration has to be performed. The deviation from linearity in the very low concentration range is caused by a slight tailing of the diflunisal peak which leads to a certain loss of signal in the automatic integration procedure. In the low concentration level (9.936 mg/l) the detected concentration was 9.06 mg/l with an absolute error —0.88 mg/l, the relative error - 8.85%. In the high concentration level 99.36 mg/l the detected concentration was 98.60 mg/l with an absolute error of —0.76 mg/l, the relative error —0.76%. On the other hand, for practical purposes it is not necessary to extend the diflunisal assay to such low concentrations since therapeutic plasma levels probably exceed the 10 mg/l concentration.

The overall recovery of the extraction of diflunisal from the plasma in this procedure is almost complete. Over the entire concentration range studied it is 96% on average, without any systematic changes.

Upon repeated measurement (10 assays) of two samples, one in the low con-

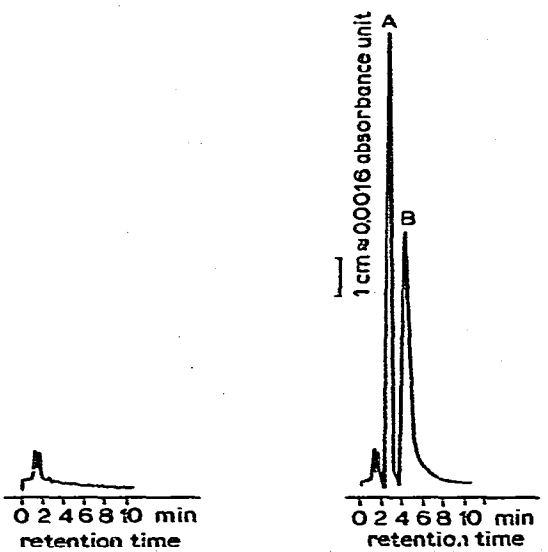

Fig. 1. Chromatogram of (left) blank plasma and (right) plasma containing diflunisal (50 mg/l) and naproxen (100 mg/l). Retention times: naproxen (A) 2.55 min, diflunisal (B) 4.11 min.

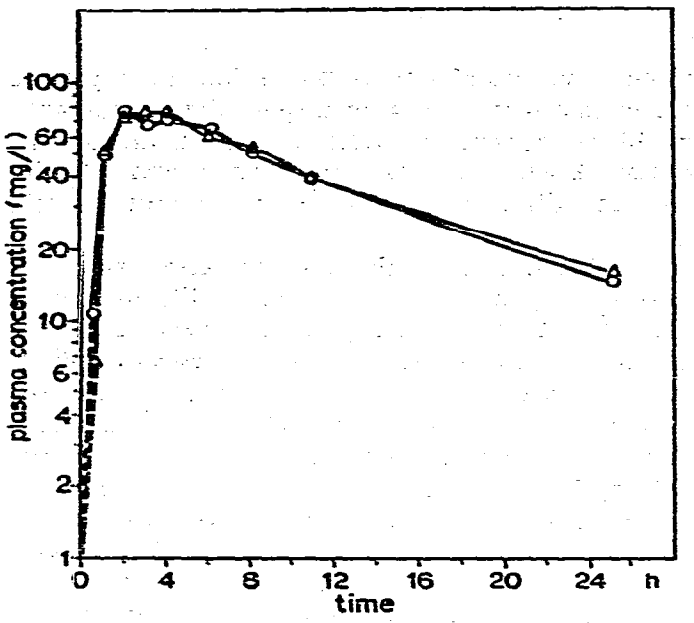

Fig. 2. Plasma curves obtained after oral administration of 500 mg diflunisal to a female volunteer, 21 years old, height 172 cm and weight 58.9 kg. △ = 2 tablets of 250 mg; ○ = 2 tablets in capsules.

centration level (9.936 mg/l) and one in the high concentration level (99.36 mg/l), a mean concentration was calculated of 9.06 mg/l with a standard deviation of 0.14, and 98.60 ± 1.66 mg/l, respectively. The relative standard deviations were 1.46% and for the high concentration 1.68%, so again no systematic changes occurred over the entire concentration range observed, with respect to the reproducibility of the method.

Currently the method is in use for the measurement of the diflunisal concentration in plasma of patients chronically medicated with the drug in the setting of a clinical trial. In connection with this trial, a preliminary study of some pharmacokinetic characteristics of diflunisal in volunteers after ingestion of a 500-mg dose either as tablets or as capsules, has been established with this procedure. The results are shown in Fig. 2. Obviously diflunisal is well absorbed, has a small apparent volume of distribution (less than 10 l) and a plasma halflife time about 10 h at the dose level applied. The method reported here is very suitable for pharmacokinetic studies and drug level monitoring of diflunisal in man.

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