Flurbiprofen, S(+), eyedrops: formulation, enantiomeric assay, shelflife and pharmacology

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Abstract
Aphatic cystoid macula edema, occurring after cataract extraction, is ascribed to trauma-induced production of intraocular prostaglandins. Sufficient experimental and clinical evidence supports the use of prostaglandin synthesis inhibitors to counteract this clinical condition. The active S (+)-enantiomer of flurbiprofen, a prostaglandin synthesis inhibitor, has been formulated into a stereoselective, ballast-free enantiomer of flurbiprofen, marketed by Syntex as enantiomeric pure NSAID, the antiinflammatory effect in eye drops (0.5%) was demonstrated experimentally [13]. In a bovine iris/ciliary body homogenate incorporating the cyclooxygenase-1 (COX-1) enzyme, the S(+) flurbiprofen proved to be the pharmacological active moiety, showing 100 times greater potency than R(-) flurbiprofen at the inhibition of prostaglandin synthesis [14].

In keeping with the benefits of only using the pro-posedly active moiety, (S+)-flurbiprofen, we investigated formulations, containing the pure enantiomers of flurbiprofen [15]. We thereby avoid isomeric bal-last, providing a reduction in metabolic load to the patient. Advantages would eventually be: less complex pharmacokinetic profiles [16], less complex drug interactions and uncomplicated concentration-effect relationships. The present study deals with the formulation, analysis, keeping quality and pharmacology of solutions containing 0.03% flurbiprofen, 0.015% flurbiprofen (S+) or flurbiprofen (R-) 0.015% all three based on their acid form.

Materials and methods
Drugs and chemicals
Flurbiprofen, (S+)flurbiprofen and (R-)flurbiprofen were purchased from Duchefa Pharma BV (Haarlem, The Netherlands). Disodiumphosphosphate.2H2O, Potassiumdihydrogenphosphate were purchased from Duchefa BV (Haarlem, The Netherlands). Water for analysis was purified in an Alpha-Q apparatus (Millipore, Bedford, MA,USA). A sample of the speciality Ocuflur® (0.03% flurbiprofen was analyzed by UV-spectrophotometry.

Conclusions
Flurbiprofen, a non-steroidal anti-inflammatory drug, has been shown to be an effective treatment for aphatic cystoid macular edema. The enantiomeric assay, shelflife and pharmacology of flurbiprofen, have been investigated.

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sodium, lotnr. 94G11 exp 01/96) was a gift from Allergan (Belgium).

Formulation of eye drops
The formulation for the 0.13M phosphate buffer, pH 7.4 and osmolality of 290 mOsmols/kg is based on previous work with indomethacin [7].

Composition of buffer solution
Disodium phosphate 2H2O 20 gram
Potassium dihydrogen phosphate 3 gram
Water for injections ad 1 liter

This solution is filtered through a 0.22 micron filter before sterilization for 15 minutes at 121 °C. This buffer solution is used for preparing the eye drops for pharmacological testing and for the shelflife procedure.

Preparation of the racemic, (S+)- and (R-)-flurbiprofen eyedrops proceeds by addition of respectively 30 mg racemic flurbiprofen and 15 mg of each of the enantiomers to 100 ml of phosphate buffer solution. The end pH remains unchanged at 7.4, because the relatively low concentration of flurbiprofen (1.2mM) does not burden the buffer (130mM) significantly. All flurbiprofen preparations were stored in glass containers. No preservative is added to the final solution because prior cataract surgery and eye-surgeons show preference to use glass containers. No preservative is added to the final formulation for the shelflife procedure.

For shelflife testing, preparations were either 0.22 micron filtered, heat treated at 100 °C for 60 minutes or sterilized at 121 °C for 15 minutes.

Preparations were stored for a period of up to 60 months, either at room temperature in subdued light or at -20 °C. Analysis was performed by capillary zone electrophoresis (CZE) on samples at t=0 and samples stored for 36, 48 and 60 months. All concentrations at the start of shelflife analysis were measured as 100%.

Analytical assay
CZE is a technique which permits high separation efficiencies combined with small sample volumes. Quantitative aspects of CZE methods for enantiomeric purity testing are discussed in the literature for both basic and acidic drugs. Depending on the resolution of the peaks, limits of detection of <0.1% are shown for determination of the minor enantiomer [17-20].

An applied Biosystems (San Jose, CA, USA) Model 270A-HT CZE system was used, equipped with a variable-wavelength UV absorbance detector (254 nm, 0.5 second rise time). The separations were performed in a fused silica capillary (70 cm x 50 μm inner diameter, Polymicro Technologies, Phoenix, AZ, USA) with a length of 50 cm to the detection window. The electrophoresis buffer was prepared by adjusting a 50mM KH2PO4 solution to pH=6.0 with a 50 mM Na2HPO4 solution. The glycopeptide antibiotic Vancomycin was used as chiral selector [21-23]. The selector was added to the inlet buffer only, at a concentration of 0.6mM.

The separations were carried out at +15 kV, with the oven temperature set at 30 °C. Samples were introduced into the capillary at the anodic end via a controlled vacuum injection system of 1 or 2 seconds corresponding to a volume of approximately 4 - 8 nanoliter, respectively. After sample injections the detector and the outer surface of the fused silica capillary were dipped for 0.5 seconds in water for cleaning. Analytes are detected in the capillary near the cathode. Data were recorded using a Fisons Model VG-Multichrom system.

Enantiomeric assay
Chiral separation occurs through selective complexation of the flurbiprofen enantiomers with vancomycin. From the corrected peak areas of the enantiomers, the enantiomeric ratio (E.R.) was calculated as R(-)/S(+). To determine if racemization occurred during storage conditions the E.R. was determined in all samples. The racemic drug will have an enantiomeric ratio of unity. For the S(+) and R(-) samples the impurities are calculated as percentage relative to the main enantiomer.

Pharmacological assay
Inhibition of prostaglandin synthesis by flurbiprofen was performed using bovine iris/ciliary body homogenate according to Van Sorge et al [14]. In brief, 25 μL of flurbiprofen solution is added to 100 μL of iris/ciliary body homogenate, prepared from one iris/ciliary body in one ml of 0.05 M TRIS buffer pH 7.4. The enzyme reaction was stopped by heating for 3 minutes in boiling water. In the supernatant after centrifugation PGE2 was determined using an enzyme immune assay. Inhibition of PGE2 release was calculated by the difference of PGE2 release in the absence and presence of flurbiprofen, expressed in percent of the non-inhibited release.

Results
Analytical analysis
Enantiomeric analysis
On injection of the phosphate buffer used in the formulation of the eyedrops no interfering peaks were detected. The E.R. of the flurbiprofen standard was...