CHAPTER 3

FLURBIPROFEN, S(+), EYEDROPS: FORMULATION, ENANTIOMERIC ASSAY, SHELF LIFE AND PHARMACOLOGY

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ABSTRACT

Aphakic cystoid macula edema, occurring after cataract extraction is ascribed to trauma-induced production of intra-ocular 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 eyedrop solution in a concentration of 0.015%. Analysis by capillary zone electrophoresis shows shelflife stability up to four years at room temperature of this enantiomer. The inhibitory effect on the synthesis of prostaglandins as measured on a homogenate of bovine iris/ciliary body, remained unaffected during a shelflife period of three years.

INTRODUCTION

Following cataract surgery non-specific inflammatory responses are induced, caused by surgical trauma. In the animal model of ocular trauma it is well substantiated that the greater part of this inflammatory process is based on synthesis of prostaglandins by iris tissue (1,2). The prostaglandins are released into the aqueous humor and cause breakdown of the blood aqueous barrier, characterized by influx of plasma proteins. Diffusion of prostaglandins through the vitreous to the posterior segment of the eye, may cause breakdown of the blood retinal barrier resulting in the development of aphakic cystoid macular edema (3).

Ophthalmic solutions of several nonsteroidal anti-inflammatory drugs (NSAIDs) are commercially available such as diclofenac (0.1%), indomethacin (0.1%), flurbiprofen (0.03%), ketorolac (0.5%) and a suspension of indomethacin (1.0%). Indomethacin 0.1% formulation was incorporated in the Dutch National Formulary (FNA) in 1986. Inpracticalities with indomethacin in aqueous solution - no sterilisation possibility and only a short shelflife when in solution (4-7) - prompted us to investigate the possibility in formulating an eyedrop based on flurbiprofen (8). An official monograph on Flurbiprofen sodium ophthalmic solution is mentioned in the USP XXIII. The solubility of flurbiprofen (pKₐ 4.22) in its acid form is 100mg/L at pH 5.0; its sodium salt has a solubility of 400mg/L (26°C, pH 7).

Shortly after it was recognized that inhibition of prostaglandin synthesis could be the main mechanism of action of NSAID’s (9), analysis of the category of 2-aryl propionic acids (profens) revealed their nature to be racemic and their inhibitory activity almost exclusively residing in the S-stereoconfiguration (10). However only in 1990 the ocular effect of topically applied S(+) ibuprofen was reported in a rabbit model of interleukin-1 (11) and paracentesis induced uveitis (12) at relatively high concentrations (0.9% and 0.8% respectively). Also with S(+) naproxen, marketed
by Syntex as enantiomeric pure NSAID, the antiinflammatory effect in eyedrops (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 in inhibiting prostaglandin synthesis (14).

In keeping with the benefits of only using the proposedly active moiety, (S+) flurbiprofen, we investigated formulations, containing the pure enantiomers of flurbiprofen (15). We thereby avoid isomeric ballast, 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). Disodiumphosphate.2H$_2$O, Potassium-dihydrogen-phosphate were purchased from Bufa (Uitgeest, The Netherlands). USP reference standard, USP 2-(4-Biphenylyl)propionic acid RS, (Flurbiprofen related compound A limit test; catalog number: 28576 ). Vancomycin hydrochloride was purchased from Sigma (St Louis, Mo, USA). H$_3$PO$_4$, KH$_2$PO$_4$, TRIS and Na$_2$HPO$_4$ were analytical grade and obtained from J T Baker (Deventer, The Netherlands).Water for injections USPXXIII (Fresenius, ’s-Hertogenbosch, The Netherlands). Water for analysis was purified in an Alpha-Q apparatus (Millipore, Bedford, MA,USA). Samples of the speciality Ocuflur® (0.03% flurbiprofen sodium, lotnumbers: 94G11 exp:01/96, 96L18 exp: 06/98 and 99K29 exp: 05/2001) were a gift from Allergan (Belgium).

Formulation of eyedrops
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 buffersolution
Disodiumphosphate. 2H$_2$O 20 gram
Potassiumdihydrogenphosphate 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 buffersolution is used for preparing the eyedrops 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 final pH remaining 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 as the eyedrops will be used prior to cataract surgery and eye-surgeons show preference to use eyedrops, if feasible, without unnecessary additions thereby avoiding possible allergic reactions.

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 complied with the requirement of containing between 90 - 110% of the active moiety and were set at 100% at the start of shelflife analysis.

Additional analysis was done, according to the monographs on Flurbiprofen mentioned in the USP24-NF19, European Pharmacopea 1999 and the British Pharmacopea 2000, on Flurbiprofen related substances of which 2-(4-Biphenylyl)propionic acid (Flurbiprofen related compound A limit test) is the main component.

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 approxi-
mately 4 - 8 nanoliter, respectively. After sample injections the electrode and the outer surface of the fused silica capillary were dipped for 0.5 seconds in water for cleansing. 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 optical isomer. The racemic samples under investigation were injected after appropriate dilution. Phosphate buffer, used in the formulation of the eyedrops, was injected as blank solution to rule out interference.

Quantitative assay
For quantitative determination of the S(+) and R(-) samples the above described system was changed for analysis of total flurbiprofen. The CZE-buffer was stabilized to pH 7 and consisted of 40mM TRIS/H3PO4 solution without chiral selector. S(+) and R(-) flurbiprofen migrate as one peak, thus minimizing the contribution of integration errors on the quantitative results. Separations were carried out at +25 kV. Samples were analyzed in duplo and on two separate days. The racemic samples and the S(+) and R(-) samples were appropriately diluted before injection. Standards were injected at the beginning, halfway and at the end of the sample sequence. Calibration was taken into account by insertion of standards in the same concentration range during the analysis. The phosphate buffer was included as blank for investigation of possible interferences.

B
A test on common impurities can be performed according to the monographs on Flurbiprofen (Sodium) described in the European Pharmacopea (1999 page 859-61), the USP24-NF19 (effective january 2000) and the British Pharmacopea (2000 page 718) To perform a limit test on 2-(4-Biphenylyl)propionic acid (Flurbiprofen related compound A), the main impurity, a liquid chromatography system was equipped with a variable-wavelength UV absorbance detector set at 247 nm and a chrompack Inertsil 5 octadecylsilyl-3 column (catalog number CP28308). As the mobile phase, at a flow rate of 1.0 ml/min, a mixture of 5 volumes of glacial acetic acid, 35 volumes of acetonitrile and 60 volumes of water was used. Injection volume used was 20 microliter with a relative standard deviation for replicate injections
of less than 1%. The resolution factor between the two principle peaks is larger than 1.5 (1.94) and accords with the requirements set by the British Pharmacopoeia, European Pharmacopoeia and USP24-NF19. The relative retention time for flurbiprofen related compound A to flurbiprofen is 0.87 (figures 1a and 1b). Sampling on several batches of flurbiprofen S(+) 0.015%, flurbiprofen R(-) 0.015% and of Ocuplu® (0.03% flurbiprofen sodium) was performed.

Figure 1a. Analysis of Ocuplu® (batch 96L18) for Flurbiprofen related compound A.

Figure 1b. Analysis of Flurbiprofen S(+) 0.015% (batch 28081995) for Flurbiprofen related compound A.
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 0.999 ± 0.006 (n=6) which corresponds to 50.0 ± 0.2%.

Stored samples of racemic flurbiprofen displayed no change in E.R. as compared to standard. Neither a heat treated sample at 100°C and stored at room temperature for 60 months (E.R.: 0.996), nor a second sample of the same date sterilized for 15 minutes at 121°C and stored at room temperature (E.R.: 0.997) showed significant deviation in E.R from the standard racemic flurbiprofen (E.R. 0.999). Samples of 48 months storage at room temperature (E.R.: 0.996) and 24 months at -20°C (E.R.: 0.995) exhibited no change in E.R. as compared to standard. Ocufur® (lotnumber 94G11) stored at room temperature (36 months) showed no change in E.R. (0.995).

All samples of S(+) flurbiprofen (48 months or less and stored at room temperature) showed no R(-) enantiomer above limit of detection of the assay, which is approximately 0.1%.

The racemic moiety of S(+) in R(-) flurbiprofen at t=0 was determined as 0.7% ± 0.1 (n=3). In Figure 2 the electropherograms are shown for two samples of R(-) flurbiprofen, after storage at -20°C for 48 months without sterilization as well as after storage for 48 months at room temperature after sterilization. The presence of S(+) flurbiprofen in the R(-) storage samples (48 months at room temperature sterilized at 15 minutes 120°C) increased to 0.9% (n=2).
Figure 2. Comparison of two superimposed electropherograms of R(-) flurbiprofen samples: (A) stored at room temp, sterilized; (B): not sterilized stored at -20 ºC.

Quantitative analysis
S(+) flurbiprofen samples irrespective of heat treatment and storage at room temperature for 48 months showed a maximum decline to 93% of declared value of the active moiety. The R(-) flurbiprofen samples revealed the same degradation characteristics as those for S(+). Ocuflu® stored at room temperature showed a decline to 92% of the declared value after 36 months while racemic flurbiprofen samples, irrespective of heat treatment showed a maximum decline to 90% in 48 months. The type of degradation reaction involved was not investigated. In the electropherogram no extra signals are detected, but in the set-up used only negative charged components with UV-absorbance at 254 nm would be visible.

Analysis of Flurbiprofen related compound A (Limit test)
Impurities can be found in compendial articles. According to the European Pharmacopoeia 3e Edition 2001 Flurbiprofen has five identified impurities. One of these is known as flurbiprofen related compound A and is used for the limit test. This substance in itself is a stereoisomer but is available as a racemic reference standard USP 2 -(4-Biphenyl)propionic acid RS.
Table 1. Flurbiprofen related compound A content in ophtalmic solutions.

<table>
<thead>
<tr>
<th>Preparation</th>
<th>Sterilized 15 min 121°C</th>
<th>Date of manufacturing</th>
<th>% Flurbiprofen related compound A</th>
</tr>
</thead>
<tbody>
<tr>
<td>S(+)Flurbiprofen 0.015%</td>
<td>Y 28-08-95</td>
<td>0,9; 1,0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>N 28-08-95</td>
<td>1,3; 1,3; 0,9; 0,8</td>
<td></td>
</tr>
<tr>
<td>R(-)Flurbiprofen 0.015%</td>
<td>Y 08-10-93</td>
<td>0,5; 0,5; 0,6; 1,03</td>
<td></td>
</tr>
<tr>
<td>Ocuflur®*</td>
<td>Y 28-08-95</td>
<td>0,2; 0,2</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Y 08-10-93</td>
<td>0,9; 0,8</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Y 28-08-95</td>
<td>0,3; 0,4</td>
<td></td>
</tr>
<tr>
<td></td>
<td>N 28-08-95</td>
<td>0,2; 0,2</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Y 94G11</td>
<td>2,5; 2,9</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Y 96L18</td>
<td>3,7; 3,6</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Y 99K29</td>
<td>2,5; 2,5; 2,6; 2,3</td>
<td></td>
</tr>
</tbody>
</table>

* Flurbiprofen Sodium. 2H₂O 0.03%. Y=yes, N=No.

In either case the products agreed with the Pharmacopoeial limits for the known impurity Flurbiprofen related compound A (5%), being stored at room temperature and irrespective of heat treatment.

In Table 1 the results are presented not only for the batches prepared with the flurbiprofen isomers as ophthalmic solution but also for the racemic flurbiprofen specialty Ocuflur®. The ophthalmic solutions of isomeric flurbiprofen have been analysed with the same maximum storage time as was stated under enantiomeric analysis.

PHARMACOLOGICAL ANALYSIS

Samples of flurbiprofen (R-) and flurbiprofen (S+) retained their inhibitory effect on prostaglandin synthesis during the period (36 months at room temperature) of shelflife analysis. This appeared from identical concentration inhibition curves using bovine iris/ciliary body homogenate (results not shown) as compared with freshly prepared solutions (Figure 3). The ratio of 100 in inhibitory potency for S(+) flurbiprofen vs. R(-) flurbiprofen also remained unchanged as appears from the unchanged IC₅₀ of 10⁻⁸ M for S(+) flurbiprofen and of 10⁻⁶ M for R(-) flurbiprofen.
DISCUSSION

The formulation of an eyedrop, like indomethacin based on a previously published phosphate buffer at pH 7.4 (7), is possible for flurbiprofen. Due to the racemic nature of the molecule and the current national and supranational policies (24) to require quality, safety and efficacy on medicinal products it was deemed necessary to exploit every possibility to formulate an eyedrop that was free of enantiomeric ballast and only contained the pharmacologically active moiety, S(+) flurbiprofen.

The first reports on use of indomethacin as an effective eyedrop to combat macular edema after cataract surgery were reassuring but were impractical in pharmaceutical sense. Short shelflife of indomethacin when in aqueous solution, uncertainty of the real available concentration when provided as a suspension and irritating when applied as an oily solution. The available specialties that have arrived on the Dutch market are not the satisfactory solution for this clinical entity. Indoptol® contains the irritating substance phenylethanol and also benzalkoniumchloride the latter which can react with indomethacin to form insoluble complexes that will remain unnoticed in a suspension. Indocollyre® incorporates the for the eye unpleasant substance methylparahydroxybenzoate and measures a high osmolality when brought in to solution (approximately 1500 mOsm/kg).
The shelflife analysis of a stereospecific ballast free flurbiprofen eyedrop formulation was carried out by CZE. To rule out pitfalls as in vitro racemization, analysis was performed of the enantiomeric ratio of flurbiprofen during the shelflife period. The observed decline in flurbiprofen was not supported by observation of degradation products in the electropherograms. This does not rule out any degradation, as only UV-active negatively charged compounds within the chosen run time would be detected. During the investigative period no evidence was found of in vitro inversion. The shelflife determination of S(+) flurbiprofen has shown the solution to be stable in glass containers for 48 months (Ocuflur® 36 months in durable plastic container) when one adheres to the limits of maximum 10% degradation. The concentration limit for the known impurity USP 2-(4-Biphenylyl)propionic acid RS has not been exceeded in any of the investigated samples. The enantiomeric inversion of R(-) flurbiprofen to the pharmacologically active S(+) flurbiprofen as observed in several species (25) has been ruled out in our assay using homogenates of bovine iris/ciliary body (14). In human blood no chiral inversion could be detected (26). Metabolism of flurbiprofen does not occur in ocular tissues as appeared from experiments in rabbits using radioactive labelled material (27).

In the pharmacological assay we investigated the potency of racemic flurbiprofen and the separate enantiomers S(+) and R(-) to inhibit the bovine iris/ciliary body cyclooxygenase-1 enzyme in producing prostaglandin E₂ (14). The S(+) moiety displayed 50% prostaglandin synthesis inhibition at a concentration of \(10^{-8}\text{M}\) (IC₅₀). Extrapolation of in vivo data on the ocular bioavailability of ophthalmic racemic flurbiprofen shows that our present data on the IC₅₀ (\(10^{-8}\text{M}\)) for inhibition of prostaglandin synthesis by S(+) flurbiprofen fall well within the concentrations of flurbiprofen, available in aqueous humor and shown to be effective in the eye after application to the outer eye (Table 2).

### Table 2. Dose/concentration relationships of racemic flurbiprofen in rabbit and human eye.

<table>
<thead>
<tr>
<th>Flurbiprofen (nmol)</th>
<th>Flurbiprofen (M)</th>
</tr>
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<tbody>
<tr>
<td>Instilled dose</td>
<td>in aqueous humor</td>
</tr>
<tr>
<td>400*</td>
<td>(800 \times 10^{-8})**</td>
</tr>
<tr>
<td>10 (ID₅₀)**</td>
<td>(20 \times 10^{-8})***</td>
</tr>
<tr>
<td>50****</td>
<td>(25 \times 10^{-9})****</td>
</tr>
</tbody>
</table>

*Values derived from (28), **average value for protein and fluorescein influx, derived from (8), *** extrapolated from (28), **** values derived from (29)
Investigation of the dose-response inhibition curves (8) reveals that racemic flurbiprofen with a concentration of 0.03% produces near maximal effect on the breakdown of the blood-aqueous barrier and this will also pertain to our stereospecific ophthalmic solution of 0.015%. In contrast to our study the flurbiprofen eyedrops used in clinical investigations or marketed specialities are composed of flurbiprofen sodium 0.03%. Flurbiprofen sodium complying to pharmacopoeial standards represents the dihydrate and therefore 0.03% is equivalent to only 0.024% flurbiprofen acid, or 0.012% S(+) flurbiprofen acid.

**CONCLUSIONS**

Analytical and pharmacological evidence is provided that eyedrops with a mere 0.015% flurbiprofen S(+) should suffice for meeting the indications for ophthalmic use and justifies its use as ballast free stereo specific drug. The shelflife of these eyedrops can be put at four years when supplied in a glass container. For a commercially available eyedrop (i.e. Ocuflur®, durable plastic eye drop container) three years is more appropriate.

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**REFERENCES**
