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A validated chiral LC method for the enantiomeric separation of naproxcinod

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ABSTRACT

A rapid isocratic chiral HPLC method has been developed for the separation of *R*-Naproxcinod from *S*- Naproxcinod. Good resolution viz. Rs > 3 between *R*- and *S*- forms of Naproxcinod was achieved with Chiralcel OD-H (250 x 4.6 mm) column using n-hexane, 2-propanol, acetic acid in the ratio of 90:10:0.5 (*v/v/v*) as mobile phase at ambient temperature. Flow rate was kept as 1.0 mL min^{-1} and the elution was monitored at 230 nm. This method is capable to detect and quantitate R- Naproxcinod to the levels of 0.14 μg mL^{-1}.

KEYWORDS

Column liquid chromatography;
Validation;
Enantiomers;
Naproxcinod.

INTRODUCTION

Separation of enantiomers has become very important in analytical chemistry, particularly in the pharmaceutical and biological fields, because some enantiomers of racemic drugs have relatively different pharmacokinetic properties and diverse pharmacological or toxicological effects[1–4]. This is one of the most vital reasons why the regulatory authorities insist more on stringent investigation for evaluating the safety and the effectiveness of drugs containing chiral centers. Enantiomeric separations have acquired importance in all the stages of drug development and the commercialization process. Therefore, the development of new methods for efficient chiral separations mainly based on HPLC, capillary electrophoresis (CE) or gas chromatography (GC) is more than necessary. Among the chromatographic methods so far developed, HPLC methods based on chiral stationary phases are widely employed for the assays of drug isomers in pharmaceutical preparations and biological fluids[5,6]. Here, we describe a robust HPLC method for separating Naproxcinod enantiomers. The method has been validated as per International Conference of Harmonization[7,8] and can be used for regular analysis in quality control laboratories of bulk drug manufacturing units.

Naproxcinod is a derivative of Naproxen with a similar anti-inflammatory activity, but less gastrointestinal toxicity. It is the first of a new class of analgesic and anti-inflammatory drugs known as cyclo-oxygenase-(COX)-inhibiting nitric oxide donators (CINODs) under development with NicOx in several countries. Naproxcinod is in phase III clinical development in the US for the treatment of osteoarthritis. The improved gastrointestinal tolerability of Naproxcinod appears to be due to its release of nitric oxide (NO) and the consequent maintenance of tissue perfusion and integrity[9].

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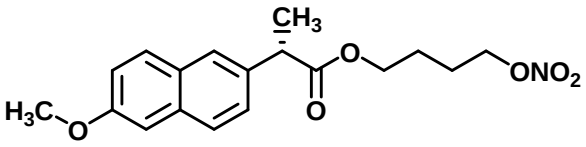
EXPERIMENTAL

Chemicals

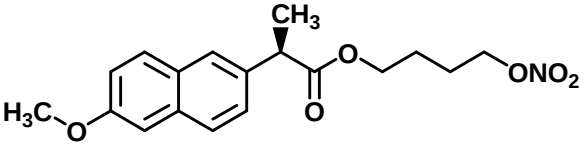
HPLC grade n-hexane and 2-propanol were procured from Merck, India. Acetic acid purchased from Standard reagents, India. *S*- and *R*- isomers of Naproxcinod were obtained from process development laboratory of Dr. Reddy's Laboratories Ltd, IPDO, Hyderabad, India and the chemical structures were given in Figure 1. The structures of potential impurities i.e. derivatives chloronaproxcinod enantiomers were given in Figure 2.

***S*-Naproxcinod:**



4-(nitroxy) butyl-(2S)-2-(6-methoxy-2-naphthyl) propanoate

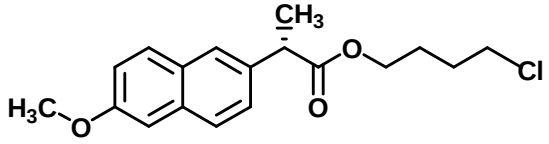
***R*-Naproxcinod:**



4-(nitroxy) butyl-(2R)-2-(6-methoxy-2-naphthyl) propanoate

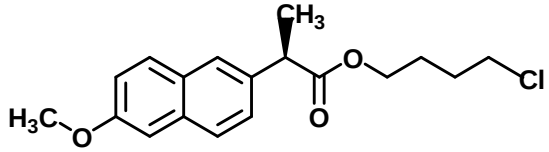
Figure 1 : *S*- and *R*- forms of Naproxcinod with chemical names

***S*-chloro Naproxcinod**



2(S)- (6-Methoxy-naphthalen-2-yl)-propionic acid-4-chloro-butyl ester

***R*-chloro Naproxcinod**



2(R)- (6-Methoxy-naphthalen-2-yl)-propionic acid-4-chloro-butyl ester

Figure 2 : *S*- and *R*- forms of Naproxcinod potential impurities i.e. chloronaproxcinod enantiomers with chemical names

Chromatographic conditions

Waters make HPLC (Alliance 2690 Model, Waters Corporation, Milford, USA) equipped with an auto sampler and 2996-photodiode array detector was used for the analysis. The data was recorded using Waters Empower software. The separations and quantification were performed on Chiralcel-OD-H column (250 x 4.6 mm, Make: Diacel Chemical Industries Ltd. Japan) with the mobile phase containing n-hexane, 2-propanol and acetic acid in the ratio of 90: 10: 0.5 (*v/v/v*) at ambient temperature. Flow rate was kept at 1.0 mL min^{-1} and the elution was monitored at 230 nm.

Preperation of solutions

1 mg each of *R*-Naproxcinod (99.00% pure) and *S*- Naproxcinod (99.50% pure) were dissolved in 10 mL mobilephase to get individual concentrations of 100 µg mL^{-1} in the mixture. This solution was used for the enantiomeric separation.

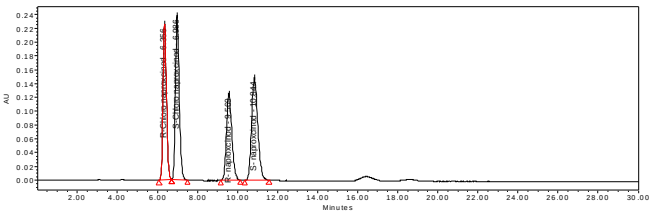
RESULTS AND DISCUSSION

Method development

To achieve separation between the Naproxcinod enantiomers, different chiral stationary phases (CSPs) were evaluated with suitable mobile phase compositions. The chiral discrimination of enantiomers occurs when they bind with the stationary phase forming transient diastereomeric complexes. The most important interactions between the analyte and the CSPs are hydrogen bonding, dipole–dipole interactions, and π–π interactions, together with the rigid structure (cellulose-based CSP) or helical structure (amylase-based CSP) of the chiral polymer bound to the support.

As naproxcinod is the derivative of naproxen, initial experiments were conducted adopting the naproxen USP chromatographic conditions. Naproxen USP chromatographic conditions comprises of mobile phase, a mixture of n-hexane:ethanol:acetic acid in the ratio of 80:20:0.5 (v/v/v) by using a column (s,s)-whelk-01[3s,4s)-4-(3,5-dinitro benzamido)-1,2,3,4-tetrahydro phenanthrene] with a flow rate of 1.0 mL min^{-1} and the chromatogram monitored at 254 nm. In these conditions the *R* and *S* enantiomers of Naproxcinod were separated. However, it was observed that *R*-chloronaproxcinod (Figure 2), one of the potential impurities, eluted together with the *R*-Naproxcinod. Trials with different mobile phase compositions did not improve the separation between the *R*-enantiomers. For example, with the mobile phase

composition of n-hexane:ethanol:acetic acid in the ratio of 90:10:0.5 (v/v/v), there was no desired separation. On the other hand, the retention times of the Naproxcinod and chloronaproxcinod enantiomers were enormously increased. Further, trials were carried out with various chiral columns and different amounts of 2-propanol as modifier to achieve the desired separation of all the four enantiomers. Chiral columns such as Chiralpak-AD-H, Chiralpak-IA (amylose based) and chiralcel-OD-H (cellulose-based) were evaluated. A good resolution of all the enantiomers was achieved by using chiralcel-OD-H column with the mobile phase of a mixture of n-hexane:2-propanol:acetic acid in the ratio of 90:10:0.5 (*v/v/v*) at 1.0 mL min^{-1} flow rate with the elution monitored at 230 nm (Figure 3). The retention times of *R* and *S* enantiomers of naproxcinod were 9.5 and 10.8 min, respectively, while the enantiomers of chloronaproxcinod eluted at 6.0 and 7.0 min respectively.



Resolution chromatogram of all enantiomers **All enantiomer spiked reference chromatogram**

Figure 3

Limit of detection and quantification

The limit of detection (LOD) represents the concentration of analyte that would yield a S/N (signal to noise) ratio between 2 to 3. LOD for *R*-Naproxcinod was found to be 0.04 μg mL^{-1} . The limit of quantification (LOQ) represents the concentration of analyte that would yield a S/N ratio between 9.5 to 10.4. LOQ for *R*-Naproxcinod was found as 0.14 μg mL^{-1}.

Linearity

The calibration curve was drawn between the peak areas of *R*-Naproxcinod versus its concentration in the range of 0.14 to 2.25 μg mL^{-1} of *R*-Naproxcinod. The slope, intercept and correlation coefficient derived from linear leastsquare regression analysis and found to be 161.37, 17.267 and 0.9999, respectively reveals that an excellent correlation existed between the peak area and concentration of the *R*-Naproxcinod.

Precision

The inter-day precision was evaluated by calculating the relative standard deviation (RSD%) of six replicate determinations by injecting six freshly prepared solutions containing *R*-Naproxcinod at two concentration levels viz. 0.14, and 2.25 μg mL^{-1} on the same day and the RSD% values were found to be 1.85 and 0.63 respectively. For intra-day precision, the sample at the above two concentrations levels was injected on six different days and the RSD% values were found to be 2.9 and 3.79 respectively. The low % RSD values confirm the good precision of the developed method.

Accuracy and specificity

The accuracy of the method was determined by spiking R-Naproxcinod to S-Naproxcinod at 0.75, 1.125, 1.5 and 2.25 μg mL^{-1} levels with three batches of S-Naproxcinod. Each determination was carried three times. The specificity defined as the ability of the method to measure the analyte accurately and specifically in the presence of components present in the sample matrix. The recovery studies of R-Naproxcinod were performed with above concentrations of R-Naproxcinod with three different batches of S-Naproxcinod by adding a sufficient amount of R-Naproxcinod to a constant amount of S-Naproxcinod sample. The prepared solutions were filtered and injected into the HPLC system. Each determination was carried out three times and the percent recovery of R-Naproxcinod was in the range of 90–110 %. In addition, the peak purities of both the forms were studied by using PDA detector and found to be homogeneous with no detectable impurities embedded in them.

Robustness

The robustness of an analytical procedure is a measure of its capacity to remain unaffected by small, but

deliberate, variations in method parameters and provides an indication of its reliability during normal usage. In the varied chromatographic conditions viz. flow rate and column temperature, the resolution between the peaks of *S*- and *R* forms of Naproxcinod was found to be 3.0 illustrating the robustness of the method.

Ruggedness

The ruggedness of a method was defined as degree of reproducibility of results obtained by analysis of the same sample under a variety of normal test conditions such as different labs, analysts, instruments and chemicals of different lots. The standard addition and recovery experiments of *R*-Naproxcinod carried out in mobile phase the above three concentration levels tested in Laboratory A were again carried out at Laboratory B using a different instrument by a different analyst. The data obtained from Laboratory B was well in agreement with the results obtained in Laboratory A, thus proving the ruggedness of the method.

Stability in solution

Standard solutions of (*R*)-Naproxcinod and (*S*)-Naproxcinod were prepared in the mobile phase at analyte concentration. Each standard solution was analyzed immediately after preparation and divided into two parts. One part was stored at 2–8 °C in a refrigerator and the other at bench top in tightly capped volumetric flasks. The stored solutions of each isomer were re-analyzed after 24 h. No change in either the chemical or enantiomeric purity was observed. The area obtained for each isomer after 24 h did not show any significant change compared with the area of initial analysis. This indicates that both isomers were stable in the mobile phase for at least 24 h when stored either at 2–8 °C or at bench top.

CONCLUSION

A rapid isocratic chiral HPLC method was developed for the enantiomeric separation *R*-Naproxcinod from *S*- Naproxcinod. The limit of quantitation is 0.14 $\mu g\ mL^{-1}$. This method has an added advantage that it not only separates the enantiomers of Naproxcinod, but also separates its most potential derivative Chloronaproxcinod enantiomers .The method is validated as per ICH guidelines and found to be linear, precise, accurate, specific, roboust and rugged for the determination of *R*- Naproxcinod in pure samples of *S*-Naproxcinod. Hence, the method can be used for the routine determination of impurities present in *S*-Naproxcinod in quality control laboratories.

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