Optimization of gas chromatographic method for the enantioseparation of arylpropionic non-steroidal anti-inflammatory drug methyl esters

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Abstract

The gas chromatography (GC) method for enantioseparation of well-known non-steroidal anti-inflammatory drugs ibuprofen, fenoprofen and ketoprofen methyl esters mixture was developed. Best enantioseparation was performed on capillary column with heptakis-(2,3-di-*O*-methyl-6-*O*-t-butyldimethyl-silyl)-β-cyclodextrin stationary phase and hydrogen used as a carrier gas. Initial temperature, program rate and carrier pressure were optimized to obtain best resolution between enantiomers.
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1. Introduction

Non-steroidal anti-inflammatory drugs (NSAID) are used for the treatment of pain and inflammation in various rheumatic and musculoskeletal disorders. One of the most important classes of NSAID is a group of 2-aril propionic acid derivatives, or "profens". Profens have been in clinical use for more than 30 years. Ibuprofen, fenoprofen and ketoprofen are widely used members of this drug class. They are marketed nowadays as a racemic mixture of active substance although S-enantiomers are significantly more potent than R-enantiomers [1–3]. Administration of only S-enantiomer will result in lower intake of the drug and less frequent side effects. However, some papers indicated that metabolism of ibuprofen and fenoprofen involves chiral inversion of the relatively inactive R-enantiomers to active S-enantiomers and no inversion is observed for ketoprofen [4–6]. Even one novel class member shows rapid chiral inversion in human plasma [7]. These facts could explain why drugs containing S-ketoprofen

as an active substance were recently introduced. Availability of S-ibuprofen also came into the focus of pharmaceutical industry. It has been shown that S-ibuprofen is characterized by less gastrointestinal toxicity than a corresponding racemic drug [3]. Moreover, some recent studies have shown that application of profen prodrugs could additionally reduce the risk of gastrointestinal injury. Among other profen prodrug types esters of arylpropionic acids have been introduced [8–13]

The active enantiomers of profens can be synthesized with excellent enantiomeric excesses by stereoselective esterification of racemic carboxylic acid using the enzyme, which react only with one enantiomer to give corresponding carboxylic acid esters. Yield of corresponding esters was analyzed by gas chromatography (GC) [14–17]. There are several papers on HPLC determination of either chiral derivatives of 2-arylpropionic acids or direct determination of 2-arylpropionic acids enantiomers [18–21]. There are also many CE methods for determination of 2-arylpropionic acids enantiomers involving different chiral buffer additives used for method optimization [22–29]. In previously mentioned papers [14,15]. authors presumed that biocatalyzed

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esterification is stereoselective and no GC chiral separation analysis on obtained esters was done. Separation of profen ester enantiomers (PEE) is of vital importance for profen enantiomer biosynthesis optimization and in-process control. Moreover, since it has been shown that profen esters are good profen prodrug candidates their analysis in pharmaceutical dosage forms, or even in biological samples is of considerable interest. In order to achieve this goal a suitable analytical method should be developed. Due to PEE volatility, low cost and speed of method development gas chromatography appears to be a more suitable choice in comparison to other separation techniques. The aim of this work was to develop and optimize a GC method for enantioseparation of methyl esters of ibuprofen, fenoprofen and ketoprofen, obtained by enantioselective biocatalytic esterification of corresponding acids and to analyze its' suitability for determination of corresponding esters. Resolution measured by tangent method was selected as a merit quantity for method optimization.

2. Experimental

2.1. Equipment and conditions

All measurements were done on AutoSystem gas chromatograph (Perkin-Elmer, Norwalk, USA) equipped with an autosampler, split/splitless injector and flame ionization detector. Turbochrom software was used for raw data analysis. Detector and injector temperature were set to 300 °C. Injection volume was 1 μL with split ratio of 1:50. Four different chiral capillaries were tested: RT-βDEXse, 30 m × 0.25 mm × 0.25 μm (Restek, Bellefonte, USA), RT-βDEXsm, 30 m × 0.25 mm × 0.25 μm (Restek, Bellefonte, USA), βDEX 110, 30 m × 0.25 mm × 0.25 μm (Supelco, Bellefonte, USA) and hidrodex β-6-TBDM, 25 m × 0.25 mm (phase thickness not mentioned) (Macherey–Nagel, Easton, USA).

2.2. Chemicals and sample preparation

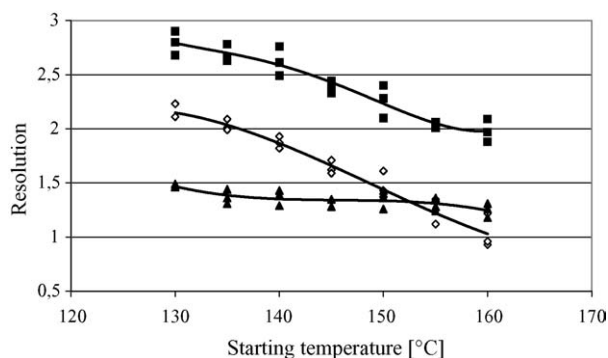Methyl esters of ibuprofen, fenoprofen and ketoprofen were prepared by esterification of in-house racemic standards of ibuprofen, fenoprofen and ketoprofen with derivatization reagent Esterate M (Supelco, Bellefonte, USA) according to manufacturers instructions. Esterifications were carried out for each drug separately by adding 10 mg of in-house standard into micro reaction vial and dissolving it with 1 mL of Esterate M. Esterification of ibuprofen was carried out on 70 °C during 1 h, ketoprofen was heated on 70 °C during 2 h and fenoprofen was heated on 70 °C overnight. After cooling down, 400 μL of each solution was transferred into 2 mL vial and injected directly into GC.

3. Results and discussion

At the beginning of the method development several critical parameters were defined: carrier gas type, type of stationary phase, sample amount, starting point of temperature program, carrier gas pressure and temperature gradient.

These variables were thoroughly examined. The column oven temperature program used before optimization was $130 \rightarrow 1\,°C/min$ up to 210 °C and carrier gas pressure was 69 kPa. The experiment started with carrier gas type selection. As the first choice He was selected as a carrier. The use of He gave zero or insufficient resolution of tested PEEs regardless of used column and other critical variables. Use of H_2 seems to be mandatory. All succeeding experiments were done with this carrier gas.
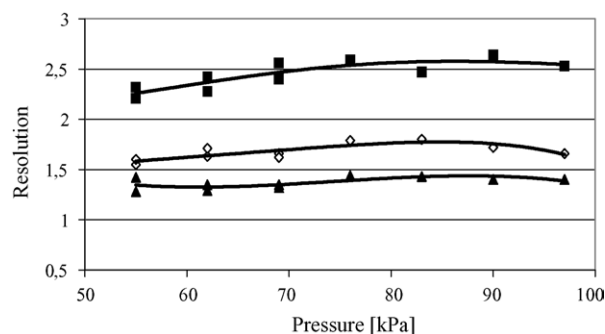

Fig. 2. Influence of the pressure on the resolution of PEE: methyl ketoprofen (▲), methyl ibuprofen (◊) and methyl fenoprofen (■).

Fig. 1. Dependence of the PEE resolution on the starting temperature: methyl ketoprofen (▲), methyl ibuprofen (◊) and methyl fenoprofen (■).
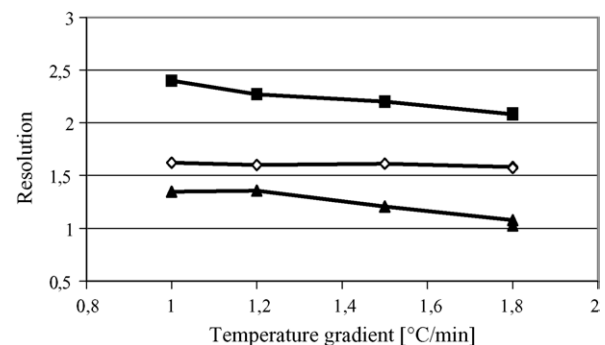

Fig. 3. Influence of the temperature gradient on the resolution of PEE: methyl ketoprofen (▲), methyl ibuprofen (◊) and methyl fenoprofen (■).
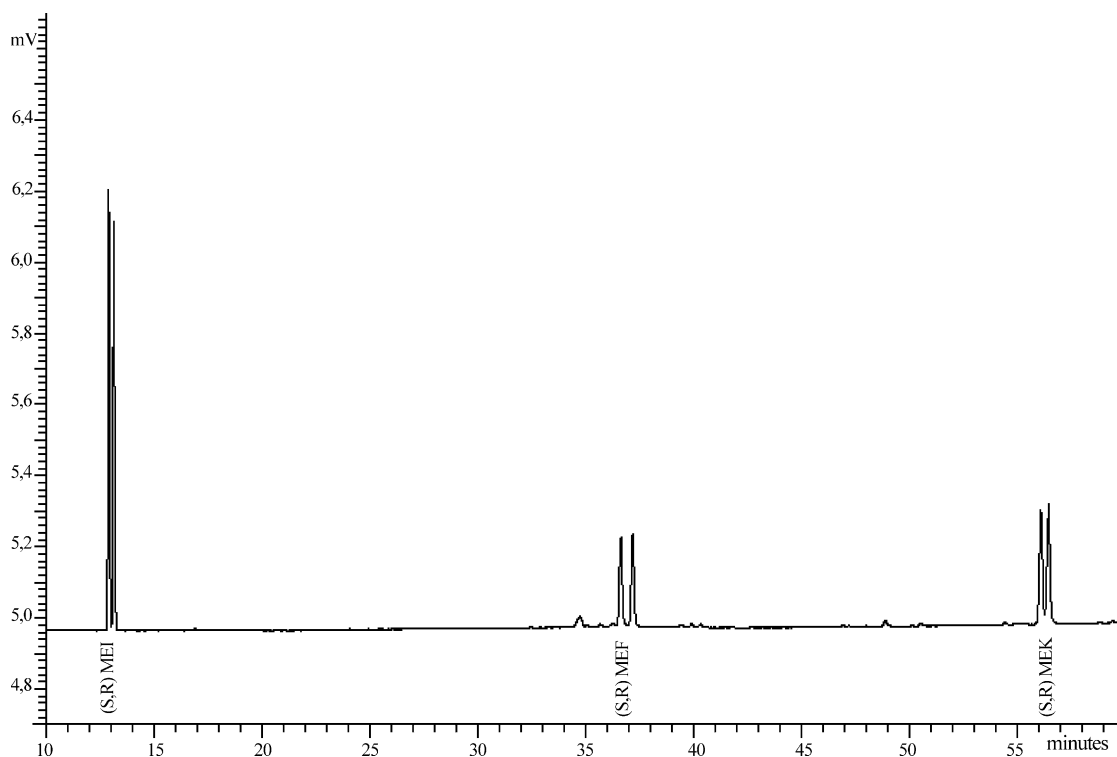
Fig. 4. Chromatogram of the final separation of PEE: methyl ibuprofen (MEI), methyl fenoprofen (MEF) and methyl ketoprofen (MEK).

All studied capillary columns have substituted β-cyclodextrins as a stationary phase. The best separation was carried out on capillary column hydrodex β-6-TBDM (Macherey–Nagel, Easton, USA), 25 m × 0.25 mm i.d. with stationary phase heptakis-(2,3-di-O-methyl-6-*O*-t-butyldimethyl-silyl)-β-cyclodextrin. Although RT-βDEXsm column has practically the same substituted β cyclodextrin stationary phase as hydrodex β-6-TBDM, resolution for all PEEs was significantly better on hydrodex β-6-TBDM column.

As the increase of sample plug length decreases resolution moderate split ratio was used (1:50). On column amount of each enantiomer was approximately 20 ng.

Starting temperature was varied by 5 °C starting from 130 to 160 °C. The first choice of the temperature gradient was 1 °C/min and the final temperature was 220 °C with carrier gas pressure of 69 kPa. Fig. 1 illustrates influence of the starting temperature on the resolution of PEEs.

It is clearly visible that an increase of the starting point temperature on the PEE results in a decrease of the resolution. Ketoprofen methyl ester resolution is the least sensitive on the starting temperature changes among all tested substances. Even for the highest value of starting temperature separation between ketoprofen methyl esters is acceptable. Since ibuprofen methyl ester enantiomers have the shortest retention times the influence of the starting point temperature is quite significant. All starting temperatures gave the highest resolution value for fenoprofen methyl ester enantiomers.

Pressure influence was tested in interval 55–97 kPa with 7 kPa steps. As a starting point temperature 145 °C was used and the temperature gradient was 1 °C/min. Fig. 2 shows that separation optimum is around 83 kPa regardless of analyzed profen molecule. In all three cases the response curve is at its maximum around this pressure. Still, the pressure influence on the resolution is weaker than the influence of the starting temperature. According to this fact pressure is a good candidate variable for decreasing the run time. By combining decrease of starting temperature and increase of pressure it is possible to speed up analysis without significant loss of resolution.

Temperature gradient influence was tested in an interval the 1–1.8 °C/min. As a starting point temperature 145 °C was used while the pressure was set to 83 kPa. Fig. 3 shows that resolution between ketoprofen methyl ester enantiomers is highly dependant on the temperature gradient while resolution between ibuprofen methyl ester enantiomers does not depend so significantly on that variable. This behavior is related to different retention times of these components.

The chromatogram obtained with column owen temperature program 145 → 1.2 °C/min up to 215 °C and with carrier gas pressure of 83 kPa is shown on the Fig. 4.

4. Conclusions

The development of the enantioselective GC method for separation of methyl esters of ketoprofen, ibuprofen

and fenoprofen has been given. Influences of the critical variables were analyzed and near optimal conditions for PEE separation were presented. Resolution measured by the tangent method in all cases was above 1 with mandatory demand for H_2 as a carrier gas. This resolution value could be accepted as a baseline separation between analyzed peaks. Therefore, it can be concluded that GC equipped with capillary containing hydrodex β-6-TBDM stationary phase represents a suitable tool for baseline enantioseparation of analyzed substances. If separation speed up is required combined decrease of starting temperature and increase of pressure is suggested. It has been shown that this operation would not compromise the enantioseparation.

Acknowledgements

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