Optimization of the separation of combretastatin A-4 cis and trans isomers by Ultra High Liquid Chromatography / Mass Spectrometry (uHPLC / MS)

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Introduction

Combretastatin A-4 (Fig. 1) belongs to a family of natural products which have the capacity to inhibit the polymerization of tubulin within cancer cells by binding to this protein. As a consequence, cell mitosis is blocked, thus inducing cell apoptosis. As the cis derivative is 30 fold more active than its trans isomer and as there is an in vivo cis to trans isomerization, a method of quantification of each compound has to be developed, aiming to determine the rate of this process. Furthermore, this method would be helpful to determine if the conditions used for the galenic formulation of CA-4, could modify the cis/trans ratio. As the structures of these two isomers were very close, the main problem was to find appropriate conditions to efficiently separate trans and cis CA-4.
Preliminary results used a Poroshell® column 120 SB-C18 with an internal diameter of 2.1 mm, a 50 mm length, particles size diameter of 2.7 µm, at a flow rate of 0.500 mL/min (injection volume of 5 µl). Moreover, the mobile phase was composed of water/acetonitrile (ACN) (40:60) (v:v).

Experimental conditions

CA-4 was analyzed using these preliminary conditions: Poroshell® column. The mobile phase was composed of a water/ACN (60:40) (v:v) mixture. Efficiency was very low and the results were insufficient in term of resolution (Fig. 2).

Consequently, the column was changed for a Zorbax Eclipse Plus® with an internal diameter of 2.1 mm, a 100 mm length, particles size diameter of 1.8 µm, at a 0.500 mL/min flow rate (injection volume of 5 µL), which significantly improved the efficiency of the column.
As both isomers of CA-4 were not totally separated, an optimization of the mobile phase was then undertaken.

The solvent system was changed to water/methanol (MeOH). After optimization, the water/MeOH mixture for the mobile phase was set to (40:60) (v:v) to 15873 and the resolution to 6.63 (Fig. 3).

Results and discussion

After the determination of suitable conditions, the optimal chromatographic system was composed of a Zorbax Eclipse Plus® column and a water/MeOH (40:60) mobile phase. This study has proven the superiority of sub-2 µm particles compared to a core-shell system: it allowed a total separation of the two isomers of CA-4, and it will be used for the rest of the pharmaceutical development of this compound.

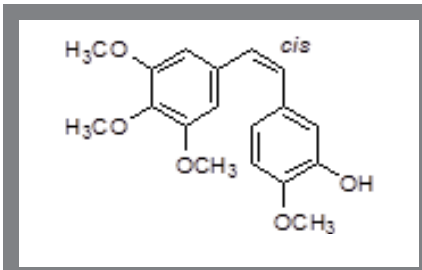

Figure. 1 – Chemical structure of combretastatin A-4 (CA-4).

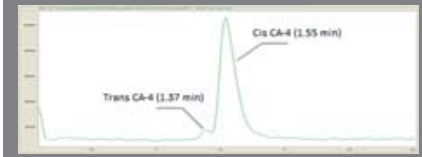

Figure. 2 – Chromatogram of CA-4 obtained with a Poroshell® 120 SB-C18 chromatographic column using a water/ACN (60:40) mobile phase.

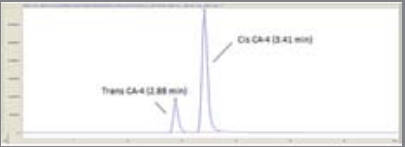

Figure. 3 – Chromatogram of CA-4 obtained using a water/MeOH (40:60) mobile phase and a Zorbax Eclipse Plus® column.

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