Developing two High-Performance Liquid Chromatography methods to study the stability of a formulated growth factor

Matthieu EYRAUD

Introduction

In regenerative medicine, the protein A, a growth factor, is used to regenerate human tissue. A biotechnology, BioChaperone, has been developed in order to improve the solubility and the stability of the protein at physiological pH. Two different analytical methods have been developed to characterize and to quantify this protein in BioChaperone formulations.
The purpose of our study was to develop two High-Performance Liquid Chromatography methods:
- A reverse-phase method (RP-HPLC) in order to quantify the intact protein and its chemical degradation products.
- A size exclusion method (SEC-HPLC) in order to quantify the total protein and its chemical aggregates.

Experimental conditions

Two analytical methods were developed and defined experimental conditions are summarized hereafter:

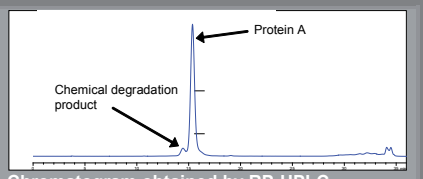
- for RP-HPLC:

Parameters	Conditions
Column	XBridge C_{18} BEH300 150x4.6 mm, 3.5 µm
Mobile Phases	Phase A : water + 0.1% TFA Phase B : acetonitrile + 0.1 TFA
Oven temperature	30°C
Injector temperature	4°C
Volume of injection	10 µl
Flow rate	0.5 mL/min
Detection	FLD (λexcitation = 280 nm, λemission = 350 nm)

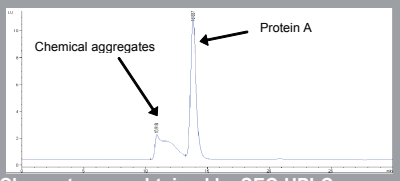
- for SEC-HPLC:

Parameters	Conditions
Column	Tosoh TSKGel 3000pWxl
Mobile Phases	$NaHCO_3/K_2CO_3$ 100mM pH 7 buffer / Acetonitrile (70/30)
Oven temperature	30°C
Injector temperature	4°C
Volume of injection	10 µl
Flow rate	0.5 mL/min
Detection	FLD (λexcitation = 280 nm, λemission = 350 nm)

Figure 1: obtained chromatograms with the two methods:



Chromatogram obtained by RP-HPLC



Chromatogram obtained by SEC-HPLC

Results and discussion

The specificity of these methods has been checked and it is good as well as the linearity and the repeatability. No interference was observed with excipients in BioChaperone formulations.
Conditions used for these two analytical methods enabled to obtain a good separation between the protein and its chemical degradation products. However, peaks resolution can be improved by modification of others parameters such as the column temperature (for the RP-HPLC). This parameter is currently under investigation and the stability of the protein at high temperature has to be studied.

Conclusion

A good separation quality between the protein and its degradation products was obtained which enabled to determine protein A concentration in BioChaperone formulations. Within the framework of stability studies, these two methods were used in order to check protein A stability in formulations. These results have been used to improve BioChaperone formulation composition.

ADOCIA
innovation for regenerative medicine
Société ADOCIA
115, avenue Lacassagne
69003 Lyon