BIO_14 - Method development for quantification of *N*-acetylneuraminic acid (NANA) in erythropoietin

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Introduction: Besides their well-known role in mediating cellular interactions, sialic acids residues are critical for biopharmaceuticals pharmacokinetics and *In vivo* therapeutic effect. *N*-acetylneuraminic acid (NANA) is the most common sialic acid and its content determination is part of quality control of erythropoietin (EPO) and meningococcal vaccine produced in Bio-Manguinhos. High-Performance Anion-Exchange with Pulsed Amperometric Detection (HPAE-PAD) provides many advantages in quantifying NANA, as it doesn't require any derivatization step nor employs organic solventes and is able to separate potential interfering compounds, as opposite to the spectrophometric method currently in place, providing a more specific quantification of NANA.

Objective: Develop a method for quantification of NANA by HPAE-PAD in the biopharmaceutical EPO produced by Bio-Manguinhos.

Methodology: The ion chromatograph 940 Professional IC Vario from Metrohm was used with the CarboPac™ PA10 column and a BorateTrap™ guard column. The mobile phase used was sodium hydroxide with sodium acetate 100 mM each. A five points standard curve was prepared within the range of 0,25 to 10,00 ppm (0,8 to 40,2 nmol/mL). After test the method and evaluate the standard curve linearity through the correlation coefficient (r) value, different hydrolysis procedures were investigated. Four conditions were tested: 0,1 N and 0,05 N trifluoroacetic acid (TFA) for 30 minutes at 80 ºC; 0,1 N hydrochloric acid for 60 minutes at 70 ºC and 2 N acetic acid for 120 minutes at 80 ºC.

Results: The linearity of the method was demonstrated through a correlation coefficient value of 0,9999. Due to the greater sensitivity of the chromatographic method, it was possibile to achieve a linear range covering smaller concentrations than the previous spectrophotometer method (8 – 64 nmol/mL). Among the conditions for hydrolysis, the one with 0,05 N TFA provided the greatest and closest value to the spectrophotometric result. Compared to the hydrolysis with the other two acids, TFA provided a higher NANA peak and a low signal from a degrated product. TFA also presented the fastest evaporation, which facilitates sample concentration after hydrolysis reaction.

Conclusion: A HPAE-PAD method using TFA for hydrolysis showed linearity and effective detection of NANA in EPO. HPAE-PAD method has many advantages against spectrophotometric method currently in place, including differentiation between NANA and *N*-glycolylneuraminic acid (NGNA), reported as immunogenic, and absence of interferences from colorimetric reaction that may led to a misreading in spectrophometric quantification. The implementation of a validated HPAE-PAD method would enhance reliability of NANA concentration results and could also determine NGNA amount in EPO as well as in other biopharmaceuticals and vaccines produced by Bio-Manguinhos.

Keywords: sialic acid; erythropoietin; HPAE-PAD