Optimization for the development of methods to quantify a protein in a biological environment by LC-MS/MS

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Introduction

One of the topics of research of the "Anabio" team in the "Institut des Sciences Analytiques", is to develop methods to quantify proteins in biological environment. This development can take several weeks, this is why an optimization of several steps of this development could save operators time. For this optimization, the team gets some robots to achieve automatically some tasks such as dilutions and Solid Phase Extractions (SPE) that are needed for the development of methods.

Material and methods

This optimization with the robot follows three steps:

- First, the program read by the robot with its software is created. For example, this program allows the robot to transfer liquid from one labware to another, to move labwares on the robot's deck, to heat, to shake labwares or to make vacuum with a vacuum pump…

- Second, an excel workbook is created to change automatically this program due to values entered into the excel workbook.

- Finally, when the program ends, the final products are analyzed by LC-MS/MS.

Analyses were made with a 4000 Q-Trap AB Sciex mass spectrometer, coupled with a LC-MS/MS system, in Multiple Reaction Monitoring (MRM) mode. This mass spectrometer uses an electrospray as ionization source and this source is heated to reach a temperature of 450K.

Results and discussion

Diluted solutions of the peptide A made by the robot were analyzed. These analyses made at a concentration higher than 5pg/mL give correct results (Figure 1). However, this is not checked for each peptide, because each one has a different response factor. So, a peptide with a high response factor could saturate the mass spectrometer at high concentrations, instead of one with a response factor which is not quantifiable at low concentrations. These dilutions were made in water so the noise was very low. However in blood or plasma this noise might be higher so the limit of quantification might be higher as well.
SPE made by the robot did not give correct results as the dilutions did. It is because these SPE do not have a good yield because a vacuum pump is used to accelerate the flow into SPE columns. If this vacuum is not well balanced it decreases the extraction yield. Therefore, analyses made after SPE by robot gave only blanks.

Conclusion

This robot allows saving time at different steps of the development of methods to quantify a protein. Dilutions gave correct results but it has not been checked with peptides into blood and it could be different. However, SPE have not given correct results yet. Tests should be performed to know the vacuum time and its value for each step of the SPE, so as the yield is increased.

Figure 1: Calibration curve of the peptide A in water between 0.005 and 10ng/mL. Dilutions made by the robot

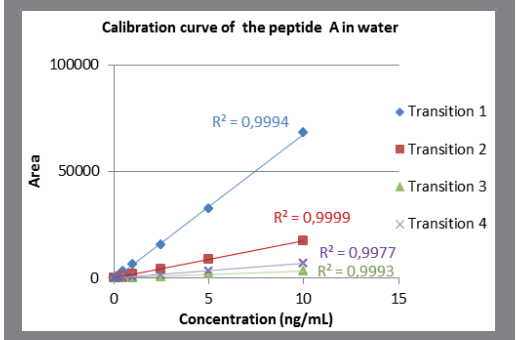
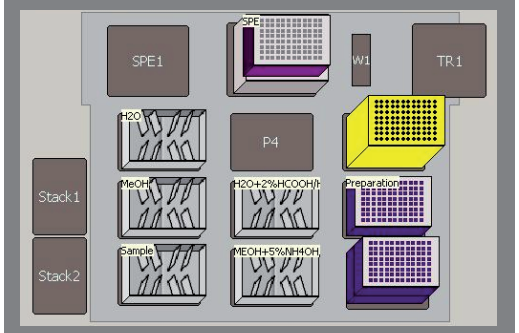


Figure 2: Example of robot instrument setup for SPE



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