

Developing a sample preparation method in SPE to quantify a protein in human urine by LC-MS/MS

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Introduction

One of the themes of the research in mass spectrometry of the team « Anabio » in the "Laboratoire des Sciences Analytiques", is to develop and validate methods to measure biomarkers in human complex matrixes (blood, urine, plasma, serum...). Urine has become one of the most attractive biofluids as it can be obtained non-invasively in large quantities and is stable compared with other biofluids. Protein P is a biomarker of renal dysfunction that can be measured in urine by LC-MS/MS.

But this protein is in a very complex matrix, and has to be purified before analysis. Solid Phase Extraction (SPE) is one of the most adapted methods to purify complex samples.

Material and method

The protein P was digested by trypsin.

Because of the ionization potential of the X-peptide ($pI = 9,9$ – as shown on figure 1), we decided to use an Oasis WCX cartridge (Waters). This cartridge is a polymeric reversed-phase, weak ion exchange mixed-mode sorbent, that allows the retention and release of strong basic compound. We can modify the pH to have a positive or negative charge on the sorbent and a positive or negative charge on the peptide.

We can also change the organic percentage to have various retentions.

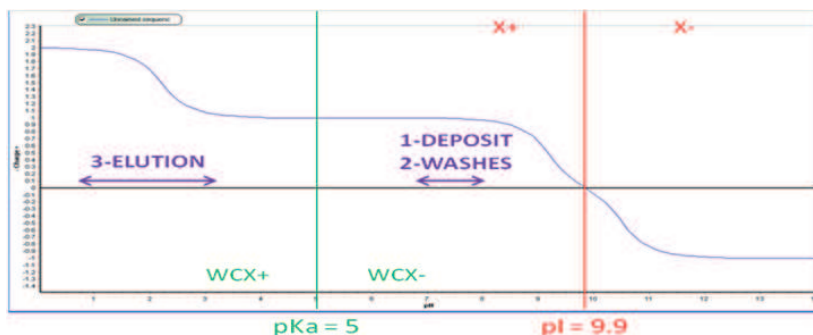


Figure 1 : potential ionization of peptide X in function of pH

We tested the influence of percentage of methanol for the wash and the elution of the X-peptide.

A solution of peptide X was put down in the cartridge at pH = 8. At this pH, the sorbent has a negative charge and X, a positive charge. So the peptide was held on the sorbent.

Washing solvent (to eliminate salts...) was composed by a mixture of 50 mM ammonium bicarbonate and methanol (pH = 8). We tested different percentages of methanol (0-20-40-60-80).

Elution solvent (to extract the peptide) was composed by a mixture of water 0.1% formic acid and methanol 0.1% formic acid (pH = 3). At this pH, sorbent has positive charge, as peptide X, and there is a repulsive phenomenon between peptide and sorbent. We also tested different percentages of methanol (0-20-40-60-80).

Washing and elution solutions were analyzed by LC-MS/MS, on an Agilent Infinity 1290 coupled with an AB Sciex Q-Trap 5500 spectrometer.

Results and conclusion

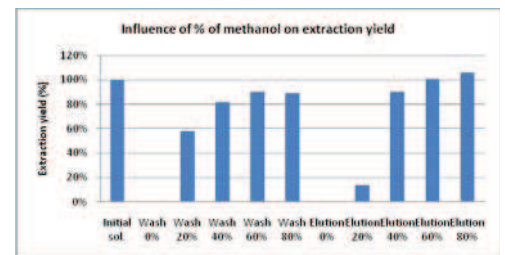


Figure 2 : influence of the percentage of methanol on SPE extraction yield

The bar chart in figure 2 showed us the results of this experiment.

For the washing part, we saw that with 20% of methanol, peptide was already extracted. As if there were no ionic interactions but only hydrophobic.

We observed that from 40% of methanol in the eluent phase, 90% of X-peptide were extracted, 100% with 60% of organic solvent.

As a conclusion, we will have to wash the cartridge at a lower pH and to be more specific, we will have to elute with a 50% methanol phase.



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