Qualitative development of Photo-SRM method peptide analysis

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Introduction

Prostate cancer is the third most frequent cancer diagnosed, it is the first in terms of number of deaths and it is associated with a high mortality rate. Various methods of detection and quantification of these diseases exist, such as the ELISA's method. There is a traditional technique using a specific antibody reagent with antigen's protein of the studied disease. However for this analysis, specific antibodies need to be developed and produced which is both time consuming and expensive. A new analytical method for complexes matrices such as biological samples has been developed, the so called Photo-SRM mode in mass spectrometry. This method allows a more sensitive analysis by adding the specificity of photo fragmentation with a laser (LID) to the selectivity of the mass spectrometry. Traditionally, the fragmentation is produced by collision with an inert gas (CID). Figure 1 shows these two SRM methods.

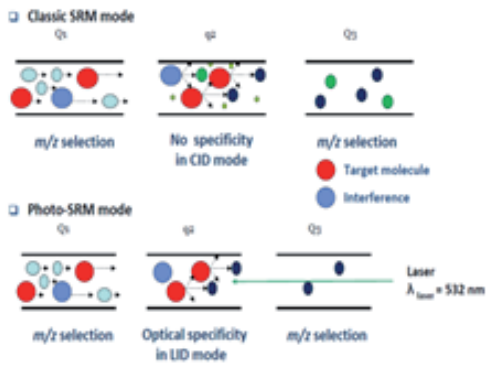


Figure 1. Comparison of two SRM methods.

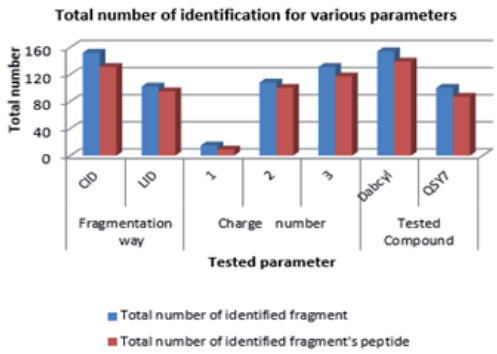
The specificity of the laser fragmentation is due to the addition of a chromophore to the targeted molecule. The selection of this chromophore is very important in the development of the photo-SRM methods. This article will discuss the choice of the best chromophore to use for the analysis of peptide from PSA (Protein Serum Albumin) in photo-SRM.

Experimental method

The studies were carried out with 4 different peptides containing cysteine residues. These peptides were derived using two different chromophores (Dabcyl C2 maleimide and QSY-7 maleimide). This preparation is realized with an addition of a fivemolar excess of TCEP (to reduce disulfure bond) and threemolar excess of chromophore in the dark during 4 hours. The derivation reaction occurs between the thiol group on the cysteine residue and the maleimide group of the chromophores. Then this sample is introduced in the mass spectrometry instrument. After the isolation and expulsion of the ion parent, a value of CID is apply to the fragmentation of this ion in Cell collision where the laser is introduced in this cell.

Results and discussion

Firstly, in order to identify the targeted molecule, we need a large coverage of sequence, i.e. a lot of specific fragment ions. With this second figure, we can see that derivation with Dabcyl chromophore makes it possible to obtain more fragment ions compare to the derivation with QSY7. Moreover, the fragmentation of larger charge state peptides also induces more fragment ions. These ions are more specific and allow the sequencing of the peptide. Concerning the fragmentation process, the LID fragmentation is better because this method offers better identification (93% in LID mode and 86 % in CID) although the signal is lower.



Tested parameter		fragment's peptide
Fragmentation process	CID	86%
	LID	93%
Charge Number	1	60%
	2	93%
	3	89%
Chromophore	Dabcyl	90%
	QSY7	87%

Figure 2. Total quantity and ratio obtained for various studied parameters

Conclusion

As a conclusion, we can say that the Photo-SRM technique using derivation with Dabcyl allow to have an easier identification and a more specific detection. This method will be used for the quantification of peptides from biological markers of prostate cancer, such as the PSA protein in real biological samples such as plasma.

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