WEBINAR: Features of Different Mass Analyzers Applied to Multi-Allergen Screening

Moderator:
Laura Bush, Editorial Director LC/GC

Featured Speakers:
Linda Monaci
Institute of Sciences of Food Production, CNR-ISPA (Bari, Italy)

Link to webinar:
Features of Different Mass Analyzers Applied to Multi-Allergen Screening

You will learn:

- how mass spectrometry overcomes traditional challenges of immunoassay-based methods
- how to improve your lab throughput and save costs
- how to improve confidence in your data by eliminating false positives and negatives

HIGHLIGHT: An evaluation of two mass spectrometry-based approaches applied to the multiple detection of egg and milk proteins in wine.

1. In the methods you described, what was your biggest challenge in terms of sample preparation for the methods?

Indeed, this is a crucial topic because we still have to work a lot in the direction to try to optimize, as much as possible, the extraction and pretreatment procedure before going to the MS detection. That's because it's very important to extract, as much possible, the proteins from the food, but also to dilute the sample to minimize an eventual matrix effect.

2. Do you have any comment on what global regulatory agencies are thinking about in terms of these analytical techniques and in terms of which allergens will become mandatory for testing?

Now, the attention is moving to the thresholds, and then I think that future efforts also from a legislative view will be aimed at setting thresholds and then also setting some limits to be respected, but it will come.

3. Could this method of testing replace all the allergen test perform in micro labs? for e.g. gluten, peanut, eggs ...

I am confident about the suitability and potential of the method that might be in future used for routine analysis once a consensus about the extraction protocol and identification of suitable markers has been reached for each matrix under study.

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4. Is there any effort to find a consensus in the scientific community as to which markers should be measured by MS for the different allergens? This would help implement the MS-based methods in routine food testing.

Some issues should be still tackled. Such issues are linked to the suitability of an extraction protocol to assure the highest extraction yield of the allergenic proteins under investigation in case of multi-target analysis. Once the consensus on this protocol is reached, suitable markers could be easily identified and monitored for developing a multi-target method.

5. In your opinion, how rapid is the adoption of mass spectrometry for allergen testing by food producers? Is it common for food producers to use this type of testing currently?

Yes, it will need still some time, but what I see in the future is a very good transition from the antibody-based recognition methods to multi-allergy detection MS-based methods because it will impact differently food producers, but also with a considerable reduction of the analysis time and cost if you take into account that, along the same analysis, you can detect a high number of food allergens at the same time.

6. Any comments about the problems of protein modification and trying to quantify the milk or egg in wines and detecting at 0.25 ppm?

Since we are dealing with detection of some peptide markers, tracing for ovalbumin, and since we chose stable markers, we don't have information about eventual modifications occurring on the native proteins. Instead, we experienced some problems in red wines where a matrix effect was evidenced that reduced sensitivity of the method. In that case, a more tailored sample prep strategy should be investigated and optimised.

7. Is it possible to detect and quantify bLG (beta lactoglobulin), which is present in the Whey fraction of milk?

Lactoglobulin could be easily detected and quantified using different MS analysers. It can be detected as intact protein or through detection of some peptide markers in a shotgun approach.

8. What internal standards are you using to adjust for the matrix effects?

At this stage we did not use any internal standard and quantification was done based on calibration curves obtained in the real matrix.

9. What do you think about advertisement phrases on the labels?

About the food labeling, I deem it very useful for the consumers but it is also necessary to have sensitive and reliable methods for their detection in the different food samples.

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10. Do you know if any regions of the world besides Europe have similar legislation regarding allergens?

I'm pretty sure that Europe is very well advanced in this field. So, I think we are at a very good point and we are now the driving force in this kind of research field.

11. How are you handling quantification? Is the inclusion of protein concentration & extraction a problem?

So far we have tested the range of linearity and compared performances of the analytical method by using two different MS platforms. We did a label free quantification by spiking the allergen free sample with increasing amounts of the allergenic proteins before extraction; then calibration curves in the real matrices were obtained correlating the MS signal to the concentration of the protein spiked in wine sample. Our results prove that, in both cases, we had very good correlation coefficients obtained and comparable LODs. Of course, this proves that the method works nicely and, once suitable peptides and transitions have been designed, the method could be applied for a fast multi-allergen screening in foods by using the untargeted High-Resolution MS approach or the SRM-MS/MS method on the linear ion trap.

12. Were the spikings done directly on the matrix or after processing of the bread/wine?

In this investigation, the spike was done on the finished product after waiting several hours to favour interaction between allergens and the food matrix. We are now performing some studies to produce allergen incurred cookies.

13. When spiking and analyzing the bread with milk and egg did you quantify and observe any milk or egg present in the bread?

The bread was allergen free and previously tested for the absence of any trace of milk and egg. In this set of experiments we just intended to check the matrix effect on the final detection of egg and milk peptides. For this reason the spike was done on the baked product after waiting a few hours to allow interaction with the matrix. We did not estimate the recovery. In this regard we are currently performing additional investigations aimed at producing incurred bread samples where the final recovery will be calculated. In this case, this will take into account the extraction efficiency and the processing applied to the food.

14. What is your recovery in your spiking experiment? And internal standard used?

In this presentation we did not show any recovery of the method in use as it was not calculated in the present work. The aim of the presentation was to show potentials of two MS analysers for multi-allergen screening in foods. Please refer to a previously published paper where we detailed these aspects for food allergen detection in wine (Monaci et al, Rapid Communications in Mass Spectrometry, 2013, 27).

15. Will internal standards reduce matrix effect? Any plan to try this in the future?

In theory, of course, a IL standard protein should be used to increase confidence in the analytical measurement, but due to the prohibitive costs, a label-free quantification could be alternatively performed.

16. Are all proteins in milk and egg allergenic? What if the targeted peptides are not allergenic?

In theory we should look for a marker of the allergenic ingredient that may give indication about the presence of that allergenic ingredient present into a food commodity. However, in this presentation, for method development our target proteins were indeed the most allergenic proteins, namely ovalbumin for egg and caseins for milk.

17. How stable is the method you described between different analysts and labs (ruggedness)?

We have not tested ruggedness of the method yet. Anyway, it is worthy to be investigated and future efforts will be directed in that direction.

18. Are there any MS-based allergen methods that have been validated in multi-laboratory studies (such as CEN or AOAC) and are accepted for allergen detection in foods by regulatory agencies in the EU or other countries?

Unfortunately, so far only in-house validations have been carried out. Discussions are underway to validate, in multi-laboratory studies, an MS-based method by using an RM.

19. You did the presence of milk in white wine at 1 ppm. Did you try 0.2 ppm in red wines (maybe a hearty burgundy)?

For red wines the method did not allow to reach such challenging LODs. In order to reach a good sensitivity also in red wines, we are currently working on the pretreatment procedure of introducing a prepurification step aiming at reducing the matrix interferences that hamper the final peptides detection. Of course, such matrix is much more complex since the abundance of polyphenols is much higher so a more tailored purification strategy should be envisaged.

20. Any comment on the applicability of triple quadrupole MS technique to this area?

Some papers have already been published on the development of an MS method for multi-allergen detection in food and are present in literature (see paper from Heick, J et al.)

21. Have you ever had the opportunity to do this experiment with a Q Exactive and compare results to your other studies?

Not for the moment, but I am really interested also in comparing results obtained on the Q Exactive that can surely improve the sensitivity of the final method. So, I am very willing to try and compare the three systems.

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22. Do you have any plans to address the matrix effects that you mentioned? Do you think they could be improved in a future experiment?

Yes, maybe by using other kinds of pretreatment routes, and also trying to work on a more selective extraction tailored to the extraction of the targeted proteins we are interested in.

23. Going back to the Q Exactive, can you comment on how the High Resolution Mass Spectrometry results could improve, particularly from a quantitative perspective, if a Q-Exactive was used since that would allow precursor ion selection? Because that would, in effect, allow you to run MRM types of experiments but with high resolving power and accurate mass, correct?

Yes, of course, this would definitely offer many additional advantages that would also translate into achieving very low and challenging limits of detection, in my opinion.

24. Is the publication available?

The paper is currently under evaluation for possible publication and, in case of interest, it will be forwarded to you upon its acceptance (contact linda.monaci@ispa.cnr.it) You can also read similar papers published: Monaci et al. Rapid Comm in Mass Spec 2013, 15; Monaci et al. Food Add and Cont 2011, 28; Monaci et al 2011 JAOAC 94.

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