Food & Allergy Consulting & Testing Services

GLUTEN METHODS TO SUPPORT CLAIMS & RISK ASSESSMENT

SUMMARY

Accurate and reliable gluten testing is imperative to ensuring the integrity of gluten-free foods.

The use of targeted proteomics by LC-MS allows direct and unequivocal identification and quantification of gluten. Because of its sensitivity, precision, accuracy, and robust quantitative ability, LC-MS provides greater assurance and protection (to both consumers and manufacturers) than other commercially available methods.

Currently, the *Regulations Relating to the Labelling and Advertising of Foodstuffs (R.146/2010)* state that R5 Mendez ELISA or other methods recommended by Codex must be used to support gluten-free claims. LC-MS meets — and exceeds — the performance characteristics required for a gluten-detection method set out in Codex. **Therefore, LC-MS complies with the regulatory requirements as a suitable method for gluten analysis.**

WHY DO WE NEED ACCURATE GLUTEN-TESTING METHODS?

The popularity of gluten-free products has increased sharply in the last decade. Gluten labelling regulations are necessary to facilitate a consistent approach to conveying information to consumers. Multiple countries have established prerequisites for gluten-free food labelling that include tolerance standards for gluten-related claims, making accurate and sensitive analytical methods imperative [1, 5].

THE EVOLUTION OF GLUTEN ANALYSES

There are many methods available for detecting and quantifying gluten, including immunological (ELISA and lateral-flow devices), genomic (PCR) and targeted proteomic (LC-MS) approaches. ELISA and lateral-flow devices are currently the most widely used testing methods. [1, 4].

Recent years have seen a dramatic change in the allergen- and gluten-testing landscape. The most prominent shift has been due to the recognition and refining of targeted proteomics (TP) as an alternative and reference method. Recent studies have confirmed and emphasised the benefits of TP, highlighting this important shift.

Want to know more?

Gluten is the collective name for a group of seed storage protein found in the endosperm of (mainly) wheat, rye, barley and oats. The cereal prolamins, which are rich in proline and glutamine, are gliadin in wheat, hordein in barley, secalin in rye, and avenin in oats [1, 2, 3].

Avenin: Although oats belong to the group of gluten-containing cereals, it has been shown that pure oats may not trigger autoimmune responses in individuals with coeliac disease. Avenin, the gluten protein found in oats, contains less proline and glutamine than gliadin, hordein an d secalin. As a result, if not contaminated with other gluten-containing grains, oats are often seen and classified as a gluten-free grain [1].

Coeliac disease (CD) is a lifelong auto-immune systemic disorder triggered by gluten found in barley, rye and wheat, and is not immediately life threatening; however, symptoms may slowly worsen and be exacerbated over days.

Gluten intolerance is a non-immune-mediated response to wheat or gluten and may not be a reaction to gluten per se, but rather to FODMAPS (Fermentable Oligo-, Di-, Mono-saccharides And Polyols) in wheat, rye or barley.

Wheat allergy is an IgE-mediated food allergy to wheat proteins, and can potentially trigger life-threatening anaphylaxes. Reactions are usually immediate, from seconds to three hours.

Gluten is a challenging substrate to measure because of the diversity of protein isomers that exist. It must also be detected in finished products that have often been heavily processed leaving the gluten protein structure modified [4].

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SHORTCOMINGS OF IMMUNOLOGICAL METHODS

Although it is the most widely used method for quantitative allergen testing, ELISA suffers from several well-documented drawbacks.

False positives (due to cross-reactivity of ELISA antibodies) lead to overestimation of risk; while false negatives (due to modification of the target epitope) lead to underestimation of risk, and expose consumers to potential reactions.

Some common food-processing methods cause partial or complete denaturation or hydrolysis of gluten. This leads to a loss of immunoreactive epitopes, resulting in the testing process underestimating how much gluten is present, or not detecting it at all [3, 7, 9, 8, 10, 11]. The need to keep proteins (including detection antibodies) in a particular conformation precludes efficient extraction procedures, compounding the risk of under-detection [2].

The different commercially available ELISA kits have been shown to respond differently to gluten from different cereals (wheat, barley and rye) [2]. This is because the target protein fraction is present in different ratios in each cereal.

Several multi-laboratory studies have been conducted in an attempt to emphasize and ensure the accuracy of ELISA results. Data suggests that although ELISAS are precise, they may not be accurate. [13]. This is particularly true of the R5 Mendez ELISA [5, 13, 3]. A case for the reliability of targeted proteomics (LC-MS).

Due to its specificity, TP by LC-MS can easily differentiate between gluten species by directly detecting multiple peptides from the proteins of interest. In addition, the advantages of MS-based methods include their ability to overcome antibody cross-reactivity and loss of gluten epitopes where ELISA-based methods are limited. This high degree of specificity provides confidence in the results, and reduces the risk of false positives and false negatives [6, 1, 18].

Previous challenges with the implementation of LC-MS for routine gluten and allergen detection included the need for specialised knowledge to operate the equipment, and lack of information on target proteins. These challenges have been overcome through widespread experimentation and publication of information on a variety of methods and targets [16, 15, 14, 17].

Given that the detection and quantification of trace amounts of gluten and allergens in food matrices is the primary goal, TP by LC-MS is the best tool for the job, given current technology. LC-MS is a great opportunity for improved food-protein analysis, on account of its sensitivity, precision, accuracy, and robust quantitative ability; these characteristics provide greater assurance to consumers and to food businesses [4].

Want to know more?

LC-MS has been used in proteomics research for over 20 years. However, until recently it has only been used routinely in commercial food testing for small-molecule contaminant detection, such as veterinary residue and pesticide detection [6].

Immunological techniques rely on the recognition and interaction of a target analyte and one or more antibodies. Antibodies recognise and bind to 'epitopes' - small portions of the whole protein molecule. The target epitopes may be linear or conformational. Linear epitopes are continuous strings of amino acids, and recognition is specific to the amino acid sequence (the primary structure of the protein). A conformational epitope may be a continuous or a discontinuous string of amino acids, and recognition depends on the three-dimensional shape of the protein (protein tertiary structure). Because antibodies only recognise epitopes, rather than the whole molecule, the specificity of an antibody depends on the uniqueness of the epitope. A lack of specificity may lead to false positives or false negatives [7, 8]. Most gluten ELISA methods target a defined section of the gluten protein. For example, the R5 assay targets epitopes with the peptide sequence glutamine-glutamine-proline-phenylalanine-proline (QQPFP), which can result in an over- or underestimation of the total gluten content [6].

Mass spectrometry (MS) is familiar to many in the food industry as a widely used analytical tool. Hyphenated methods such as LC-MS/MS, coupling a separation technique with MS, allow the direct and absolute identification and quantification of allergens. Mass spectrometric methods are routinely performed at the peptide scale, making detection independent of the tertiary structure of the allergen, extremely specific, and still detectable after food processing. [12, 9, 8].

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REGULATORY & CODEX REQUIREMENTS

The *Regulations Relating to the Labelling and Advertising of Foodstuffs (R.146/2010)* recommend that R5 Mendez ELISA or other methods recommended by Codex are used to support 'gluten-free' claims.

Codex standard 118/1981 ('Foods for special dietary use for persons intolerant to gluten') refers to immunologic methods (ELISA), but makes provision for the use of other methods provided they have at least equal sensitivity and specificity. Additionally, it sets out performance characteristics for gluten detection methods:

- They must show sensitivity and specificity equal to immunologic methods.
- They must react with or target the cereal-protein fractions that are toxic for persons intolerant to gluten.
- They must not cross-react with other cereal proteins, constituents of foods, or ingredients.
- They must be validated and calibrated against a certified reference material, if available.
- Their detection limit must be appropriate, according to the state of the art and the technical standard.
- The detection limit should be 10mg gluten/kg or below.
- The qualitative analysis indicating the presence of gluten must be based on relevant methods.

DOES THE GLUTEN LC-MS METHOD COMPLY WITH CODEX S PERFORMANCE CHARACTERISTICS?

Yes, it complies with the Codex performance characteristics. See the table below for more information:

PERFORMANCE CHARACTERISTICS	LC-MS CAPABILITY
Sensitivity & specificity	Superior sensitivity and specificity as described in the above sections.
Protein target	The LC-MS method employed by FACTS targets a series of peptides from the following proteins: B1-hordein, B3-hordein, avenin-3, 75k gamma secalin, glutenin subunit DY 10 and PW212, Beta-amylase, alpha-amylase inhibitor 0.19.
Cross-reactivity	Cross-reactivity is not a known challenge associated with the LC-MS technique. The method exclusively targets peptides from the above proteins; therefore, it is not cross-reactive with other cereal proteins or constituents of food or ingredients.
Validated	The method has been comprehensively validated to accurately and precisely detect and quantify gluten in a wide range of food matrices.
Calibration	No certified reference material exists currently. FACTS uses wheat, barley and rye flour as calibrants.
Limit of detection (LOD)	The LOD is currently set at 1ppm, lower than the 10ppm performance criterion.
Method relevance	The relevance of LC-MS as a gluten detection and quantification method is well documented in the literature. This is discussed in the sections above.

CD Coeliac disease
LC-MS Liquid chromatography-mass spectrometry
PCR Polymerase chain reaction
MS Mass spectrometry
ELISA Enzyme-linked immunosorbent assay
TP Targeted Proteomics

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