QUANTITATIVE ANALYSIS OF GMO IN FOOD AND FEED: DIGITAL PCR VERSUS REAL TIME PCR METHOD

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The European legislation establishes that food and feed are labelled for their Genetically Modified Organism (GMO) content when the ingredients contain authorized GMOs and provides a tolerance thresholds of 0.9% for authorized GMOs in Europe and 0.1% (only in animal feed) for GMOs authorized in third countries. Therefore, the ability to quantify nucleic acids with accuracy and precision is fundamental to comply with EU legislation.
Currently, the most widely used technique to detect the presence of GMOs in food and feed is the real-time PCR that requires a standard curve to calculate the quantity of an unknown target sequence. Recently, the digital PCR was used as an alternative to real-time PCR in different analytical fields, including GMOs, given the major benefits that have been found in its use: absolute quantification free from standard curves and amplification efficiency of the reaction; high precision metrological use; high sensitivity in the detection of rare events; no inhibition due to the matrix effect; possibility to perform analysis in duplex and multiplex, so as to reduce the amount of reagents and the global analysis costs.
The present work consists in the validation of the method for the quantitative analysis of GM maize MON 810 and the assessment of applicability of the digital PCR method (absolute quantification using sealed-chip technology) in samples of highly processed food and feed. During the digital PCR validation process, a quantitative analysis of the same reference material samples was simultaneously conducted using the real-time PCR platform. With the purpose of avoid biases when comparing the two platforms, we transferred the validated MON 810 specific assay for Real Time PCR to digital PCR with minimum adaptation (primers, probe, DNA concentration were the same). Linearity of the response, the limits of detection and quantification, trueness and repeatability of assays complied with international recommendations [1, 2]. The applicability with different matrices and the practicability of use for routine GMO testing were also evaluated. From the obtained results, it was assessed that the two methods were completely comparable even when the digital PCR method didn't exceed the real-time PCR performance.
The digital PCR showed high performance and demonstrated to be a solid analysis method and the analytic data obtained from the work highlighted: high precision on low sample concentration levels; high analysis accuracy; applicability of the method on complex and highly processed matrix; transferability of validated real-time PCR methods to the digital PCR platform without major protocol changing; lower running costs than those of the standard quantitative PCR technology (it was assessed an average cost per sample of 15.67 Euros for digital PCR analysis and 19.75 Euros for real-time PCR analysis).Hence, it was concluded that digital PCR can be applied for routine quantification of GMOs, or any other field where quantitative analysis is required.

[1] Codex Committee On Methods Of Analysis And Sampling (2010) Guidelines On Performance Criteria And Validation Of Methods For Detection, Identification And Quantification Of Specific DNA Sequences And Specific Proteins In Foods. Available: http://www.codexalimentarius.org/download/standards/11667/CXG_074e.pdf.
[2] European Union Reference Laboratory for GM Food and Feed (2008 October) Definition of minimum Performance requirements for analytical methods of GMO testing. Available: http://gmo-crl.jrc.ec.europa.eu/doc/Min_Perf_Requirements_Analytical_methods.pdf.