SIXTH FRAMEWORK PROGRAMME

GM and non GM supply chains: Their CO-EXistence and TRAceability

Outcomes of Co-Extra

Validation of novel methods and technologies

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There is a continuous and increasing need for reliable analytical methods to assess compliance with national and international requirements in all areas of analysis. The reliability of a method is determined by the so-called validation, which is the procedure providing evidence of suitability of an analytical method for its intended purpose. Based on the results of a validation study, a method is considered or not as fit for the intended purposes. In most cases, formal validation requires the assessment of the performance of the proposed method by means of an inter-laboratory study, also known as collaborative study or ring trial.

While well established validation key parameters and data analysis procedures are available for single-target qualitative and quantitative methods (see for example ISO 5725 and http://gmo-crl.jrc.ec.europa.eu/doc/Min_Perf_Requir_Analyt_methods_131008.pdf), the validation of novel methods and technologies developed by the Co-Extra project required the development of novel approaches to summarise the information provided by individual validation indices and tests statistics into comprehensive indicators of method performance.

With the aim of providing easy access to statistical and numerical tools for analytical method validation, the freely available Analytical Method Performance Evaluation (AMPE) software was created. Through AMPE, a variety of validation metrics (indices and test statistics) is provided for comparing measurements from a laboratory analysis and reference values from standard samples. Provisions are also provided for analyses based on blank samples. In its innovative part, AMPE supplies provisions for fuzzy-based aggregation of validation metrics.

Through the application of fuzzy logic, aggregated indicators are proposed as suitable tools for overall evaluation of analytical methods, allowing also objective comparison across different methods. Fuzzy-logic based indicators were developed that allow summarising the information

obtained by independent validation statistics into one synthetic index of overall method performance. The possibility of having a comprehensive indicator of method performance has the advantage of permitting direct method comparison, facilitating the evaluation of many individual, possibly contradictory metrics. In its original development, the fuzzy-based expert system was used to validate novel methods developed by the project, the DualChip® GMO microarray and the so-called pJANUS plasmids, and to test the "modular approach" to method validation.

The DualChip® GMO is a novel multiplex screening method for the detection and identification of GMO, based on the use of multiplex PCR followed by microarray. The technology is based on the "Matrix Approach" i.e. on the identification of quite ubiquitous GMO genetic target elements first amplified by PCR, followed by direct hybridisation of the amplicons on a predefined microarray. The validation was performed within the framework of Co-Extra, in collaboration with twelve laboratories. The method was evaluated with predefined performance criteria with respect to the JRC-IHCP CRL-GMFF method acceptance criteria. Data were processed according to ISO 5725 standard and the overall method performance met the acceptance criteria. However, creating reproducible data with a high level of consistency across array experiments and various platforms is widely accepted by the scientific community as a major issue. The complex nature of a microarray experiment results in many potential sources of variability, which can affect performance. In response to this challenge, the fuzzy-logic based approach was successfully applied to the analysis and data interpretation of the chip validation exercise.

A second method was developed, based on real-time PCR and the use of novel calibration molecules (plasmid pJANUS) in comparison to genomic DNA calibrant (the classical approach). The method was validated among various laboratories and the evaluation of the inter-laboratory study performed indicated that the dual-target plasmid pJANUS™-02-001 can be used as calibrant in determining the amount of Roundup Ready® soybean line GTS-40-3-2. The equivalence of plasmid and genomic DNA templates as calibrants for the quantification of the GM soybean event GTS-40-3-2 was demonstrated: this equivalence was assessed through conventional statistical analysis (ANOVA) and with the application of the fuzzy-logic based approach, that resulted remarkably in line with the expert interpretation of statistical results; this can be seen an a further validation of the suitable application of the novel fuzzy logic analysis in GMO detection methods evaluation.

A study was performed to provide a proof-of-concept for the application of the modular approach to analytical methods in the field of GMO testing based on the use of the Real-time PCR. For general information on the modular approach refer to M. Van den Bulcke presentation, "Modular Approach Implemented: Pros, Cons and Future Perspectives".

Modularity implies independency and thus flexibility of combining modules on the one hand, and uniformity and harmonisation on the other hand. If modular validation is to be applied, fit for purpose procedures and general acceptance of minimum requirements for each module are needed in order to evaluate the uncertainties associated with each module. In order to provide scientific evidence on the applicability of this approach, the experiments carried out focused on the interactions between DNA extraction methods and PCR analysis. It was found that for the correct application of the modular approach appropriate performance criteria should be met by DNA extracts (assessment of quality characteristics of DNA) so that they can be fit for the purpose of the following analytical module, independently from the preceding matrix-DNA extraction combination. With the exception of one DNA extraction/matrix combination, the study provided good evidence of independency of the analytical modules tested, suggesting that a modular approach can be correctly employed in method validation and analytical control.