

THE MAIZE TRUST

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Methodology for the analyses of

(i) Multi-mycotoxins in maize;

(ii) Fumonisin mycotoxins in maize

During 2016 the Maize Trust appointed a committee and funded a project to evaluate the efficacy of multi-mycotoxin analyses performed by the two main mycotoxin analytical laboratories in South Africa, i.e. the Mass Spectrometry Unit of the Central Analytical Facility (CAF) of Stellenbosch University (SU), and the Southern Africa Grain Laboratory (SAGL). In a second inter-laboratory study quantification of the fumonisin B mycotoxins by LC-MS and HPLC methods were evaluated, by including CAF (SU), SAGL, Cape Peninsula University of Technology (CPUT), and the Agricultural Research Council-Grain Crops Institute (ARC-GCI).

Committee:

Dr JF Alberts (CPUT; project leader)

Prof. G Shephard (CPUT)

Dr MA Stander (SU)

Ms H Meyer (SAGL)

Co-workers:

Dr B Janse van Rensburg (ARC-GCI)

Ms P Mngqawa (CPUT)

Background

Analytical methods for reliable and accurate determination of mycotoxin levels are of fundamental importance in determining the extent of mycotoxin contamination in maize and maize products during the production, storage and processing stages. Chemical analysis of mycotoxins in South Africa is performed by a number of laboratories, using a range of screening, semi-quantitative or fully quantitative HPLC-based methods, which meet the requirements of various local industries or exporters. However, very few can undertake the type of multi-mycotoxin analytical

methods by HPLC with tandem mass spectrometry, which are currently being adopted in many laboratories of the developed world and among South Africa's trading partners. As this is a relatively new technique, it was regarded by the Maize Trust of the utmost importance that the technique be evaluated in terms of accuracy, repeatability and sensitivity, to ensure precision of analysis.

The aim of the project was to identify and address factors that could negatively impact on accurate analyses of multiple mycotoxins in maize, i.e. extraction methods, analytical standards, efficacy of quantifying the respective mycotoxins and interpretation of results.

Outcome

Several factors that negatively affect precision of results were identified and recommendations were made. The study resulted in standardisation of extraction and quantification methods between laboratories as well as recommendations to ensure sustainable high precision of analyses.

Recommendations

The recommendations should be implemented by all laboratories/analysts performing extractions/analyses of multiple mycotoxins (and fumonisins) in maize.

- **Matrix vs. solvent standards:** The results indicated that overall more acceptable % recovery levels were obtained with matrix-matched standards.

Matrix-matched standard calibration curves should be used by both laboratories. SAGL supplies control maize for preparation of maize extract.

- **Extraction solvent and method:** It was decided to standardise the extraction methods between the two laboratories for analyses of Maize Trust project samples:

The two laboratories will use the same extraction methods and solvents for multi-mycotoxins and fumonisins from maize. It was decided to use extraction solvent H₂O: methanol: acetonitrile (50:25:25).

- **Quality Control measures (Important):**

To ensure precision of results, a reference maize sample (e.g. certified samples from FAPAS, Biopure, etc.), containing known levels of the relevant mycotoxins, should be included in each LC-MS run.

- **Reporting of results (e.g. publications and progress reports):**

The following information should be included:

- % Recovery.
- LOD and LOQ values.
- R² values.
- It was suggested that % RSD values should be included in tables rather than “standard deviation” values.

- **Reporting of results: units presented in result reports**

It was noted that some result reports received from analytical laboratories did not include the units of measurements. It is of extreme importance that researchers should verify units used and follow-up where units are not presented in reports. Incorrect units/absence of units can lead to misinterpretation of results.

- **Reporting of results: µg/kg and ng/ml vs. ppb**

Results should preferably be presented as, for example, mg/kg, µg/kg and ng/ml; not ppm or ppb.

- **Training**

General concern expressed:

The general handling of analytical standards by researchers is a concern. In certain cases, researchers provide their own standards for analyses and concern was expressed on the preparation of standards by researchers with no training or experience in this field.

It was stressed that all analysts/researchers performing extraction and HPLC or LC-MS quantification analysis should undergo training.

Training on extraction methods, preparation of analytical standard solutions and quantification of multiple mycotoxins by LC-MS and HPLC is offered by CAF, SU and SAGL.