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Advanced and Relevant Methods for the Determination of Mycotoxins in Food and Feed

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Abstract

Mycotoxins are primarily low molecular weight, polar organic compounds that are soluble in a variety of organic solvents and are generated by fungal secondary metabolism. Mycotoxins have a significant impact on both animal and human health. The purpose of this article is to highlight the use of different chromatographic separation techniques in the determination of mycotoxins in food and feed. The significant implications for human and animal health of the ingestion of these mycotoxins and the ensuing legislative requirements in many countries necessitated the development of analytical methods. High-performance liquid chromatography (HPLC) has become the predominant separation technique in mycotoxin analysis, while thin-layer chromatography and gas chromatography are still utilized. UV or fluorescence detectors have found widespread use in mycotoxin determination, even though HPLC has become the omnipresence method of choice. These systems either make use of the mycotoxin of interest's inherent UV absorption or fluorescence, or they derivatize it using techniques that have been developed to allow for suitably sensitive detection.

Thin-layer Chromatography (TLC)

Since 1961, TLC has been the traditional method used for the analysis of mycotoxins in food and feed. Over time, with increased data accuracy requirements, separation and quantification procedures have been improved from TLC to HPLC. Although in the past most determinations of AFB_1 were made by TLC methods, the percentage of their use has decreased significantly in recent years. However, TLC methods remain recommended for the detection of aflatoxins in plant materials due to their low cost and lower equipment demand. Aflatoxins separated on silica gel plates can be easily visualized under UV light, and TLC methods can be applied for the screening of mycotoxins in raw materials. Specific confirmatory methods have also been developed for certain mycotoxins separated on TLC plates, and possible interferences can be distinguished by using a sulfuric acid spray. Another variation of TLC is the use of OPLC, which integrates the benefits of HPLC and TLC.

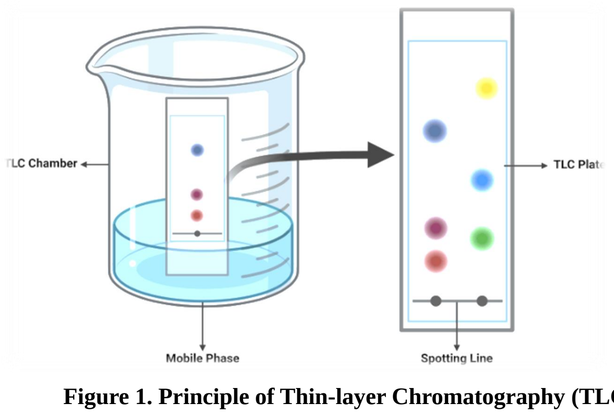

Figure 1. Principle of Thin-layer Chromatography (TLC)

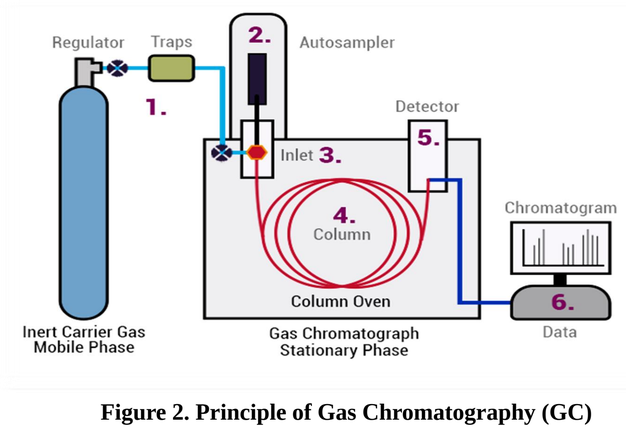

Figure 2. Principle of Gas Chromatography (GC)

Gas Chromatography (GC)

Gas chromatography is rarely used in mycotoxin analysis because mycotoxins are non-volatile and polar substances, requiring a derivatization step to convert them to volatile derivatives. This is usually achieved by silylation or acylation which occurs after purification. Among electron capture detectors (ECD), flame ionization (FID), and single mass spectrometry (MS), the latter is the most widely used in GC analysis. Analyzers such as the ion trap and the quadrupole have also been used. The time-of-flight (TOF) analyzer was used to analyze TCs in wheat. In addition, the triple quadrupole (QqQ) in GC-QqQ-MS/MS technique was presented by Rodríguez-Carrasco for the analysis of TC, PAT, and ZEA in wheat semolina.

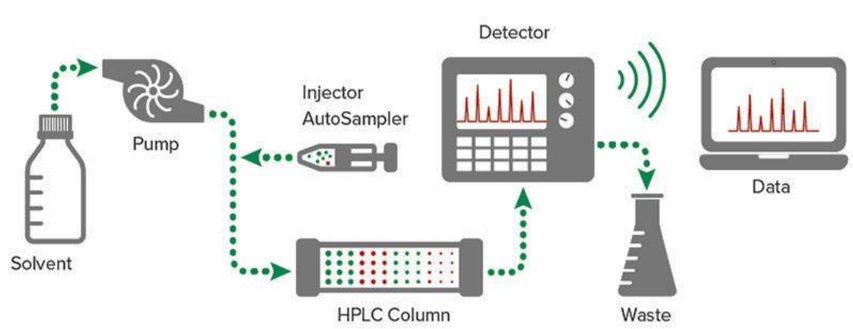

Figure 3. Principle of High Performance Liquid Chromatography (HPLC)

High Performance Liquid Chromatography (HPLC)

The choice of analytical technique depends on the nature of the mycotoxin, and HPLC offers high-resolution and state-of-the-art automation. Detectors used in HPLC mycotoxin analysis include fluorescence (FLD), UV-visible (UV), photodiode array (PDA), and MS (single mass spectrometry and tandem MS (MS/MS)). HPLC with FLD or UV detectors can be used to determine chemically related mycotoxins. HPLC with FLD is used for OTA quantification due to its reliability and sensitivity, without the need for a chromophore due to natural fluorescence. For other types of mycotoxins, such as FB, derivatization is necessary because they do not have chromophores in their structure. HPLC-FLD is useful for the analysis of AF, ZEA, and DON. HPLC with PDA is used to detect ATs, and an HPLC-PDA-FLD system can simultaneously determine AF, DON, OTA, and ZEA in wheat bran.

Mass spectrometry (LC-MS/MS)

The LC-tandem MS (MS/MS) technique is essential for the accurate analysis of mycotoxins at trace levels, being considered modern, sensitive and reliable compared to HPLC. The European Committee for Standardization has recently published an official methodology for the analysis of mycotoxins such as ZEN, T_2 and HT_2. Studies have shown that LC/MS-MS is effective in the determination of multimycotoxins in various matrices such as cereals, beer or drugs.

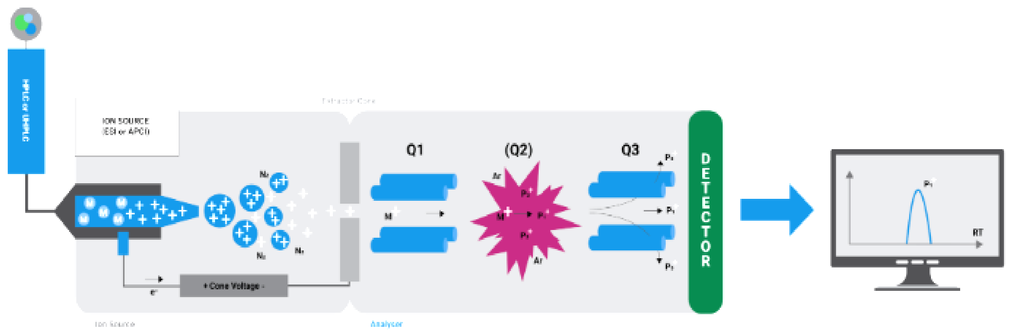

Figure 4. Principle of Mass spectrometry (LC-MS/MS)

Immunochemical methods (ELISA)

Rapid diagnostic methods such as ELISA, strips, flow membranes, and LFD are important for the detection of mycotoxins. ELISA is used for the rapid detection of mycotoxins, through the interaction of the antigen-antibody complex with chromogenic substrates and the spectrophotometric measurement of the color developed. ELISA tests can detect AF, ZEA, OTA, DON, T_2/HT_2, and FB in various agricultural products. LFDs are rapid screening tools used for immunochromatographic assays and can be used to obtain positive or negative results. However, limitations include interferences and the complexity of analyte identification matrices.

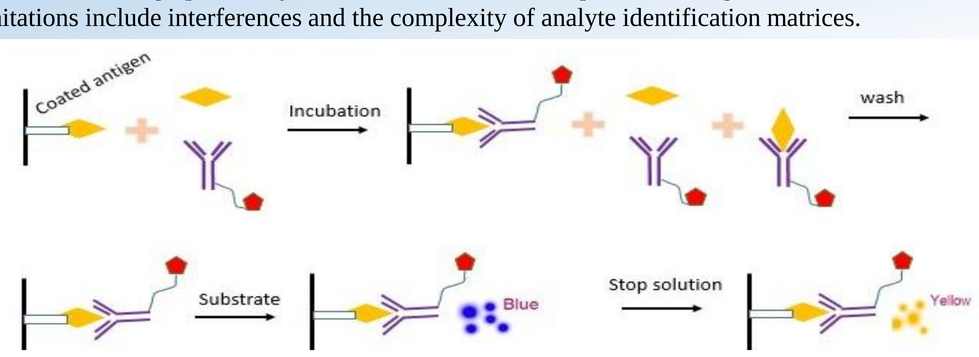

Figure 5. Principle of Immunochemical method (ELISA)

Conclusions

Mycotoxins are responsible for food contamination and there are certain permissible limits for them, which is why it is important to develop sensitive and reliable methods for their detection. Before detecting and quantifying mycotoxins in contaminated samples, different extraction and cleanup protocols are applied. Sample preparation techniques should reduce analysis time, use small volumes of solvents, and have adequate extraction scale. A fundamental tool for the analysis of several mycotoxins in the same matrix is LC/MS-MS, due to its high sensitivity, accuracy, and reliability. The use of chromatography is limited due to the cost of equipment, the need for specialized personnel, and complex protocols. Immunoassay-based methods such as rapid immunoassays and biosensors are recommended for rapid and on-site analysis.