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Foodstuffs – Determination of fumonisins B1 and B2 in maize – HPLC method with solid phase extraction clean-up

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English version

Foodstuffs - Determination of fumonisins B1 and B2 in maize - HPLC method with solid phase extraction clean-up

Produits alimentaires - Dosage des fumonisines B1 et B2 dans le maïs - Méthode CLHP avec purification par extraction en phase solide

Lebensmittel - Bestimmung von Fumonisin B1 und B2 in Mais - HPLC-Verfahren mit Reinigung durch Festphasenextraktion

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Foreword

This European Standard has been prepared by Technical Committee CEN /TC 275, "Food analysis - Horizontal methods", the secretariat of which is held by DIN.

This European Standard shall be given the status of a national standard, either by publication of an identical text or by endorsement, at the latest by June 2002, and conflicting national standards shall be withdrawn at the latest by June 2002.

The annexes A, B and C are informative.

According to the CEN/CENELEC Internal Regulations, the national standards organizations of the following countries are bound to implement this European Standard: Austria, Belgium, Czech Republic, Denmark, Finland, France, Germany, Greece, Iceland, Ireland, Italy, Luxembourg, Netherlands, Norway, Portugal, Spain, Sweden, Switzerland and the United Kingdom.

1 Scope

This European Standard specifies a method for the determination of fumonisin B_1 (FB_1) and fumonisin B_2 (FB_2) in maize using high performance liquid chromatography (HPLC).

The method has been successfully validated in an interlaboratory study according to AOAC Guidelines for Collaborative Studies [1] on maize containing 405 µg/kg to 6732 µg/kg fumonisin B_1 and 152 µg/kg to 2619 µg/kg fumonisin B_2. The method works well with maize or minimally processed maize (e.g. fresh, dried and milled maize), but does not provide reliable results with most maize-based processed products.

2 Normative references

This European Standard incorporates by dated or undated reference, provisions from other publications. These normative references are cited at the appropriate places in the text, and the publications are listed hereafter. For dated references, subsequent amendments to or revisions of any of these publications apply to this European Standard only when incorporated in it by amendment or revision. For undated references the latest edition of the publication referred to applies (including amendments).

EN ISO 3696 *Water for analytical laboratory use - Specification and test methods (ISO 3696:1987).*

3 Principle

Fumonisins are extracted from the sample of maize with a mixture of methanol and water. The filtered extract is purified on a strong-anion-exchange (SAX) solid-phase extraction (SPE) cartridge, and the fumonisins are eluted with a mixture of acetic acid and methanol. The extract is evaporated and the residue is redissolved in methanol and o-phthaldialdehyde/2-mercaptoethanol (OPA/MCE) is added to form fluorescent fumonisin derivatives. The derivatives are analysed by reverse-phase high performance liquid chromatography (HPLC) with fluorescence detection.

WARNING - Fumonisins are hepatoxic, nephrotoxic and carcinogenic to rats and mice. Effects on humans are not fully known. Observe appropriate safety precautions for handling fumonisins. Any laboratory spills should be washed with a 5 % solution of sodium hypochlorite.

4 Reagents and materials

4.1 General

During the analysis, unless otherwise stated, use only reagents of recognized analytical grade and only distilled water or water of grade 1 as defined in EN ISO 3696. Solvent shall be of quality for HPLC analysis.

4.2 Methanol, abs.

4.3 Methanol solution, volume fraction $\varphi(CH_3OH) = 75$ %

Mix 75 parts per volume of methanol (4.2) with 25 parts per volume of water.

4.4 Acetonitrile solution, $\varphi(CH_3CN) = 50$ %

Mix 50 parts per volume of acetonitrile with 50 parts per volume of water.

4.5 o-phosphoric acid, $\varphi(H_3PO_4) = 85$ %

4.6 Acetic acid-methanol solution, $\varphi(CH_3COOH) = 1$ % for eluting the SPE column.

Mix 1 part per volume of glacial acetic acid with 99 parts per volume of methanol (4.2).

4.7 o-phthaldialdehyde (OPA)

4.8 2-mercaptoethanol (MCE)

4.9 Sodium dihydrogen phosphate solution, substance concentration $c(NaH_2PO_4 \cdot 2H_2O)$ = 0,1 mol/l

Dissolve 15,6 g of $NaH_2PO_4 \cdot 2H_2O$ in 1 l of water.

4.10 Disodium tetraborate solution, $c(Na_2B_4O_7 \cdot 10\ H_2O)$ = 0,1 mol/l

Dissolve 3,8 g of $Na_2B_4O_7 \cdot 10H_2O$ in 100 ml of water.

4.11 Sodium hydroxide solution, c(NaOH) = 0,1 mol/l

Dissolve 0,4 g of NaOH in 100 ml of water.

4.12 Mobile phase

Mix 77 volume parts of methanol (4.2) with 23 volume parts of sodium dihydrogen phosphate solution (4.9). Adjust to pH 3,35 with o-phosphoric acid (4.5). Filter the solution through a 0,45 μm membrane (5.7) filter.

The mobile phase composition may have to be adjusted to conform with individual HPLC column characteristics.

4.13 Derivatization reagent

Dissolve 40 mg of OPA (4.7) in 1 ml of methanol (4.2) and dilute with 5 ml of disodium tetraborate solution (4.10). Add 50 μl of MCE (4.8) and mix. The reagent solution is stable for up to one week at room temperature in a dark and capped amber vial.

4.14 FB_1 and FB_2 standard solution

Prepare individual stock solutions of FB_1 and FB_2 at mass concentrations of 250 μg/ml in acetonitrile solution (4.4). Commercially available standard solutions may be used. Transfer 100 μl aliquots of each stock solution to a glass vial and add 300 μl of acetonitrile solution to yield 500 μl of a standard solution containing both fumonisins at individual mass concentration of 50 μg/ml. If a calibration curve is made, mix different aliquots of standard solutions (i.e. 20 μl, 50 μl, 100 μl and 200 μl) of individual fumonisins and dilute to 500 μl with acetonitrile solution to obtain the relevant calibration solutions.

Fumonisin standard solutions are stable up to at least six months when stored at approximately 4 °C.

5 Apparatus

Usual laboratory equipment and, in particular, the following:

5.1 HPLC apparatus, comprising the following

5.1.1 High performance liquid chromatograph, isocratic pump set to deliver e.g. 1 ml/min constant flow rate, equipped with an injection system capable to deliver e.g. 10 μl.

5.1.2 Analytical reverse-phase separating column, e.g. octyldecylsilane (ODS), which ensures a baseline resolution of the fumonisin peaks from all other peaks, with the following characteristics:

— stainless steel;

— a length of 150 mm;

— an inner diameter of 4,6 mm;

— a stationary phase with particle size of 5 μm;

— a suitable corresponding reverse-phase guard column.

Columns of other dimensions can also be used.

5.1.3 Fluorescence detector with the capability of using excitation wavelength of λ = 335 nm and emission wavelength of λ = 440 nm.

5.1.4 Data system

5.2 Blender, homogenizer, or mixer.

5.3 Fluted filter paper

5.4 Strong-anion-exchanging solid phase extraction column, e.g. Bond-Elut ® [1)] SAX-cartridges, containing 500 mg of sorbent, or similar has been found to be suitable.

5.5 SPE extraction manifold

5.6 Solvent evaporator, with heating module, or similar.

5.7 Membrane filter, for aqueous solutions, with a pore size of 0,45 μm.

6 Sampling

It is important that the laboratory receives a sample which is truly representative and has not been damaged or changed during transport or storage.

7 Preparation of the test sample

Grind the sample to pass through a 1,0 mm sieve and homogenize the sample.

8 Procedure

8.1 Extraction

Place 50 g of the test sample into a suitable glass or plastic container (e.g. a 250 ml plastic centrifuge bottle). Extract for 3 min with 100 ml of methanol solution (4.3) using the blender (5.2) at approximately 10 000 min^{-1}. The time needed for complete extraction can vary with the type of equipment used.

Centrifuge the blended extract for 10 min at 500 *g* and filter the supernatant through a fluted filter paper (5.3). Check the pH of the eluate and adjust, if necessary, with sodium hydroxide solution (4.11) to between pH 5,8 and pH 6,5.

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[1] Bond-Elut® is an example of a suitable product available commercially. This information is given for the convenience of users of this European Standard and does not constitute an endorsement by CEN of this product.

8.2 Clean up

Attach the SPE cartridge (5.4) to the SPE manifold (5.5) and condition by washing successively with 5 ml of methanol (4.2) and then with 5 ml of methanol solution (4.3). While maintaining a flow rate of no more than 2 ml/min, apply a 10 ml aliquot of the filtered sample extract to the SPE cartridge. Wash the SPE cartridge with 8 ml of methanol-water solution (4.3), followed by 3 ml of methanol (4.2). Do not allow the cartridge to run dry. Discard the washings. Elute the fumonisins with 10 ml of methanolic acetic acid 1 % (4.6) at a flow rate not more than 1 ml/min. Collect the eluate in a suitable vial.

Transfer the eluate in the collection vial to a suitable vial and evaporate the eluate to dryness by using the solvent evaporator (5.6) under nitrogen at approximately 60 °C. Wash the collection vial with 1 ml of methanol (4.2) and add the washing to the suitable vial. Evaporate the additional methanol to dryness under nitrogen, ensure that all the acetic acid has evapored, and cap the vial. Retain the dried residue at approximately 4 °C until HPLC analysis, that should be performed within two weeks.

8.3 Derivatization and determination

8.3.1 Standard derivative solution

Transfer 25 µl of the fumonisin standard solution (4.14) to the base of a small test tube. Add 225 µl derivatization reagent (4.13), mix the solutions vigorously, and inject an aliquot (e.g. 10 µl = 0,050 µg FB_1 and FB_2) of the derivatized solution onto the HPLC column (5.1.2) at a reproducible time within 3 min after addition of the derivatization reagent. If a single point calibration is used, adjust the sensitivity of the fluorescence detector (5.1.3) to at least an 80 % recorder response.

8.3.2 Maize test solution

Redissolve the dried residue (see 8.2) in 200 µl of methanol (4.2). Acetonitrile solution (4.4) may also be used. Transfer 25 µl of this solution to the base of a small test tube and add 225 µl of the derivatization reagent (4.13). Mix the solutions and inject an aliquot, e.g. 10 µl of the derivatized solution onto the HPLC column (5.1.2) at a reproducible time within 3 min after addition of the derivatization reagent (4.13). If fumonisin chromatographic peaks exceed those of fumonisin standard solution or exceed the highest point of the calibration curve (4.14), make additional dilutions of the sample extracts with methanol (4.2) and repeat derivatization.

NOTE 1 It is critical to adhere to reproducible times between the addition of the reagent (4.13) and the injection onto the HPLC column, because of progressive decay in fluorescence of the fumonisin-derivatives that occurs after periods in excess to 4 min.

NOTE 2 Limits of detection and quantification vary considerably according to the sensitivity of the detector used. Limits of detection (signal/noise = 3) as low as 5 µg/kg can be reached with the new generation of fluorometric detector.

NOTE 3 A typical chromatogram is given in annex A.

9 Calculation

Calculate the mass of each fumonisin m_{ti} in micrograms, injected onto the HPLC column using equation (1):

$$m_{ti} = m_{si} \times \frac{P_t}{P_s} \qquad (1)$$

where

P_t is the fumonisin peak of the test sample, in units of height or area;

P_s is the fumonisin peak of the standard solution, in units of height or area;