Application of planar chromatography on determination of sterigmatocystin in foods

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Introduction

Sterigmatocystin is a toxic metabolite structurally closely related to the aflatoxins, and consists of a xanthone nucleus attached to a bifuran structure. Sterigmatocystin is mainly produced by the fungi Aspergillus nidulans and Aspergillus versicolor. The occurrence of sterigmatocystin in raw materials and foods has been reported often. The instances reported have usually been on mouldy, or poor quality materials such as wheat, malling barley, maize, animal feed, hard cheese, pecan nuts and green coffee beans. It appears to occur much less frequently than the aflatoxins, although analytical methods for its determination have not been as sensitive until recently, and so it is possible that small concentrations in food commodities may not always have been detected.

Sterigmatocystin has usually been determined by multitoxin analysis in conjunction with other mycotoxins. The most frequently analytical methods used are thin-layer chromatography (TLC and HPTLC) and high performance liquid chromatography (HPLC). The less in use is method of gas chromatography (GC).

Experimental

This presentation deals with the development of planar chromatography method for determination of sterigmatocystin in chosen foods.

Sample characterization

- 20 samples of malt - raw material for beer production
- 10 samples of Edam cheese - sampled in the Czech retail

Preparation of standards

A standard solution (cca 20 µg/mL) of sterigmatocystin in benzene was prepared from the crystalline substance (Sigma S-3255) according to AOAC method 970.44 and 976.22. Concentration of the standard solution was determined by absorbance measurement at absorption maximum λ = 320 nm.

The standard solution was stored in a refrigerator (-20 ºC) and was used to spike samples and was diluted to obtain working solutions (usually 1.0 µg/mL) of sterigmatocystin for preparing calibration curves.

Analytical methods

Chromatography was performed on 20 cm x 10 cm silica gel RP-18 WF 2545. HPTLC plates. Diluted calibration standard (1, 5, and 10 µL corresponding to 1, 5, and 10 ng sterigmatocystin), and cleaned samples were applied by spot technique with a Camag model III automatic TLC sampler; spots were 1 cm from the edge of plate, distance between samples was 5 mm. Prechromatographic derivatization with trifluoroacetic acid (2 µL) was used. The plates were developed with acetonitril-isopropanol-0.25 N H₃PO₄ (4:5:10, v/v), in the dark, in a saturated 20 cm x 10 cm vertical development chamber. After drying in a stream of cold air the plate was sprayed with solution of iron (III) chloride in ethanol (20%) and heated (105 ºC) for 10 min.

Sterigmatocystin was measured by fluorescence densitometry by means of a Camag TLC Scanner II with mercury lamp and K 400 secondary filter. The excitation wavelength was 366 nm, the emission wavelength 420 nm, and the SENS and SPAN parameters were 160 and 50, respectively. The Rf value of sterigmatocystin under these conditions was 0.15. Validation of the method was performed according to the principles of the ICH Guideline for pharmaceutical analysis.

Results

The study was performed to produce selective, sensitive and accurate HPTLC method for determination of sterigmatocystin in selected foods. Linearity, limit of detection, limit of quantification, recovery and repeatability were tested. The relationship between peak area and the amount of sterigmatocystin applied, in a specified working range 1.0 - 10 ng per spot, was determined in Fig. 4. The correlation factor was 0.997. The limit of detection (LoD) of sterigmatocystin was found 0.9 µg/kg and the limit of quantification (LoQ) was found 3.0 µg/kg in malt and Edam cheese. The recovery was tested by running recovery experiments with spiked samples of malt and Edam cheese. The mean recovery was 85-90 % in the range 1 - 50 µg/kg of selected foods. The repeatability was tested in accordance with CSN ISO 3534-1. The results are shown in Table 1.

<table>
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<th>Table 1. Results of repeatability experiments</th>
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<td>Samples of malt naturally contaminated with sterigmatocystin</td>
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Conclusions

In summary, an HPTLC method for quantification of sterigmatocystin in cereal grains (barley, malt) and hard cheese was developed. It is sensitive to levels of 3 µg/kg (limit of quantification). The method is useful for inspection of cereals after harvesting, and it is able to use its modification for determination of sterigmatocystin in other matrices.