Application of planar chromatography on determination of sterigmatocystin in foods

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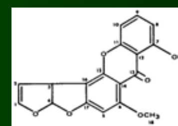
Introduction

Aspergillus nidulans

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 not been reported often. The instances reported have usually been on mouldy, or poor quality materials such as **wheat**, **malting 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 gass chromatography (**GC**).



Sterigmatocystin

Experimental

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

Sample characterization

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

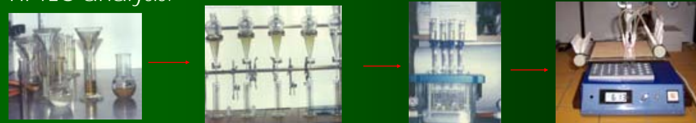
Preparation of standards

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 °C**) and was used to spike samples and was diluted to obtain working solutions (usually **1.0 μg/mL**) of sterigmatocystin for preparing calibration curves.

Extraction and clean-up procedure

The ground **sample** (20 g) was blended with addition of **chloroform** (80 mL) at high speed. The solution was filtered and evaporated to dryness under vacuum and then dissolved in **methanol** (18 ml) with addition of **4% NaCl** (2 mL). The extract was transfered to the separation funnel, defatted with **n-hexan** (10 mL) and sterigmatocystin was extracted to **chloroform** (2*20 mL). The **solid-phase extraction** on **silica-gel columns** (500 mg) was used for cleaning procedure. Sterigmatocystin was eluted with **benzene-ethylacetate** (8:2, *v/v*; 20 mL). The purified extract was evaporated to dryness at first under vacuum and then under nitrogen, and dissolved in **benzene** for HPTLC analysis.



Analytical methods

Chromatography was performed on 20 cm x 10 cm **silica gel RP-18 WF 254S** 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 H_3PO_4** (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 °C) 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 R_F 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 with linear response, was determined in Fig. 4. The correlation factor was **0.997**. The **limit of detection** (LoD) of sterigmatocistin 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.

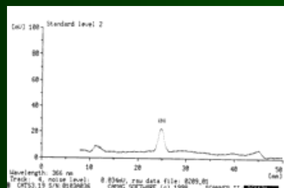


Fig. 1 Densitogram obtained from sterigmatocystin standard [5 ng per spot]

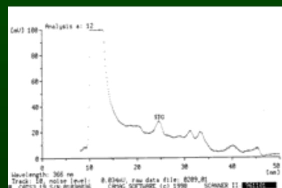


Fig. 2 Densitogram obtained from malt sample [12 μg per kg]

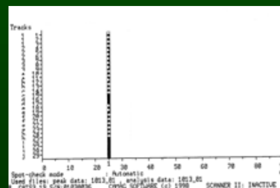


Fig. 3 The R_f value of sterigmatocystin

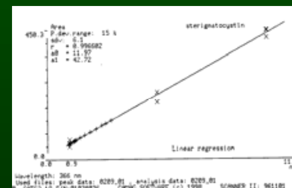


Fig. 4 Calibration function of sterigmatocystin

Table 1. Results of repeatability experiments

	Samples of malt naturaly contaminated with sterigmatocystin
n	10
x (μg/kg)	9. 91
SD (μg/kg)	1. 811
Repeatability (%)	18.28

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 usefull for inspection of cereals after harvesting, and it is able to use its modification for determination of sterigmatocystin in other matrices.

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