ELABORATION OF SOLID PHASE EXTRACTION METHOD FOR ANALYSIS OF STERIGMATOCYSTIN

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Abstract

Sterigmatocystin is a mycotoxin produced by fungi of many *Aspergillus* species and it is a biogenic precursor of aflatoxin B_1. For analysis of various mycotoxins to clean up sample extracts, mainly solid phase extraction (SPE) is used. An elution of sterigmatocystin from Strata X and Strata C18 SPE columns by different acetonitrile-water and methanole-water solutions were cheked in this paper.

Acquired results showed a possible suitability of both columns for the analysis of sterigmatocystin.

Keywords: Sterigmatocystin, mycotoxin, solid phase extraction, C18 columns, Strata X.

Introduction

Sterigmatocystin (STC) is a mycotoxin produced by fungi of many *Aspergillus* species. Sterigmatocystin is a biogenic precursor of aflatoxin B_1 (Hsieh et al., 1975; Betina, 1998) (Fig.1.) and is known to be produced by isolates of *Aspergillus versicolors, Aspergillus nidulans* (Atalla et al., 2003), and *Bipolaris sorokiniana.* Schroeder and Kelton (1975) reported that a large number of isolates of *Aspergillus parasiticus* and *Aspergillus flav*us produce STC, and they also added *Aspergillus chevalieri, Aspergillus ruber,* and *Aspergillus stelodami* to the list of known STC producers.

Since mycotoxins are normally present in food and food products at very low levels, a strong concentration of the extract is necessary to make detection possible. The frequent presence of lipids and other substances that may interfere in the final detection makes it necessary to clean up the extract prior to concentration by column clean up and/or precipitation of impurities. Several chromatographic clean up steps are possible with materials such as silica gel, modified silica gel, aluminium oxide, polyamide, Florisil®, and Sephadex®. Silica gel is most frequently used sorbent. Prepacked columns are now commercially available. Many recently published analytical methods for mycotoxins use these columns. The advantages of such prepacked columns, e.g. Sep-pak® and Baker®, are obvious. Time, needed to prepare the columns is saved, and variations in preparation of columns between analyses are less. On the other hand, variations between lots of prepacked columns have been reported (Van Egmont et al., 1986), and they do not offer the possibility of easily introducing slight variations in the column composition (for instance, adjustment of the water content or column size). The sample extract is usually added to the column in an appropriate solvent, after which the column is washed with one or more solvents in which the toxins are insoluble or less

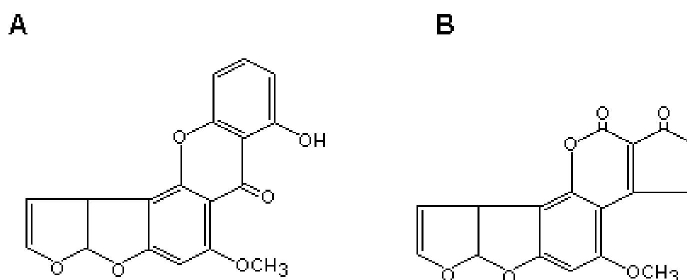

Figure 1. Chemical structures of (A) sterigmatocystin and (B) aflatoxin B_1.

soluble than the impurities. Then the solvent composition is changed in such a way that the toxins are selectively eluted from the column and the eluate is collected.

Today the most popular clean up SPE columns for mycotoxins are based on normal and reversed phase chromatography, as well as ion-exchange (Jestoi et al., 2004; Uhlig et al., 2004; Vesonder et al., 1985).

For analysis of STC by thin layer chromatography (TLC), sample extracts basicaly are purified by Florisil (Vesonder et al., 1985) and phenyl-bonded SPE columns (Stroka et al., 2004).

Strata X SPE column contains a new polymeric sorbent – styrene divinylbenzene. It is developed for the reversed phase (RP) SPE of polar and non-polar molecules.

Strata C18 SPE column contains a classic C18 sorbent for SPE .

During this study, Strata X and Strata C18 SPE columns for the first time were applied for the analysis of STC.

The aim of this study is to compare two different STC eluting procedures (using acetonitrile-water solutions and methanol-water solutions) with two different SPE columns Strata C18 and Strata X.

Materials and Methods

Strata C18 (500 mg) SPE column (Phenomenex Co., Torrance, USA) and Strata X (500 mg) SPE column (Phenomenex Co., Torrance, USA) were used for the experiments.

Chemicals and reagents

Methanol (HPLC-grade) and acetonitrile (HPLC-grade) were purchased from 'Merck' (Darmstadt, Germany). Deionized water was purified with Millipore Milli-Q Plus system (Millipore, Molsheim, France). Sterigmatocystin (STC) standard was purchased from 'Sigma' (St Louis, USA). Argon (AGA, Latvia) was used as a collision gas in the mass spectrometry.

Preparation of standard solutions

Stock solution A (500 μg ml^{-1}) preparation: 5 mg of solid STC standard was dissolved in 10 ml of acetonitrile.

Working standard solution B (10 μg ml^{-1}) preparation: 200 μ l^{-1} of stock solution A was dissolved in 20% water solution in acetonitrile.

Working standard solution C (10 μg ml^{-1}) preparation: 200 μ l^{-1} of stock solution A was dissolved in 20% water solution in methanol.

Experiment

During the experiment, possibilities of STC holding and eluting options from the Strata X and Strata C18 SPE columns were checked.

Column conditioning procedure: column was conditioned with 6 ml of methanol, followed by 6 ml of water prior to use.

STC loading into the column and eluting from the column was done using acetonitrile-water system:

5 ml of working standard solution B were loaded in Strata X SPE column. The outgoing eluate was the first control fraction, then the column was progressively washed with 5 ml portions of pure eluting solution which consisted of different composition of acetonitrile-water. Eluting solutions composition was (% volume/volume): 25/75, 30/70, 35/65, 40/60, 45/55, 50/50, 55/45, 60/40, 70/30 and as the final eluting solution – pure acetonitrile.

The second control fraction was outgoing eluting solution 25/75, the third fraction was outgoing eluting solution 30/70 until the last 11th fraction which consisted from a pure acetonitrile.

The collected eluting solutions fractions were analyzed for content of STC by Liquid Chromatography – tandem Masspectrometry (LC – MS/MS).

STC loading in the column and eluting from the column was performed using methanol-water system:

Column conditioning procedure was the same as described above.

5 ml of working standard solution C were loaded in Strata X SPE column. The outgoing eluate was the first control fraction, then the column was progressively washed with 5 ml portions of pure eluting solution which consisted of different composition of methanol-water. Eluting solutions composition was (% v/v): 25/75, 30/70, 35/65, 40/60, 45/55, 50/50, 55/45, 60/40, 70/30, and as the final eluting solution – pure methanol.

The second control fraction was outgoing eluting solution 25/75, third fraction was outgoing eluting solution 30/70 until the last 11th fraction which consisted from a pure methanol.

The collected eluting solutions fractions were analyzed for content of STC by LC – MS/MS.

The experiments on Strata C18 columns were followed by the same procedure.

LC-MS/MS – analysis

A Waters Alliance 2695 liquid chromatograph (Waters Co., Milford, MA, USA) was connected to a MicroMass Quattro LC triple-quadrupole

mass spectrometer (Micromass Ltd., Manchester, UK). An electrospray ionization (ESI) probe in the positive mode was used in the analysis of sterigmatocystin. The mobile phase consisted of 0.01% formic acid in acetonitrile and 0.01% formic acid in water (75:25) used in isocratic regime. The column used was a Phenomenex Luna $C_{18}(2)$ (5 μm), 3.0 x 150 mm (Phenomenex Co., Torrance, USA). The flow rate was 0.3 (ml min^{-1}) and the injection volume was 50 μl. The parameters of the mass spectrometer were optimized using the sterigmatocystin standard. The best response was recorded with the following parameters: cone voltage – 30 V, capillary voltage – 3.5 kV, extractor – 2 V, RF lens – 0.2 V, source temperature – 120 °C and desolvation temperature – 350 °C, cone gas flow – 63 (l h^{-1}), desolvation gas flow – 553 (l h^{-1}), and collision energy 30 eV.

For the structural identification in MRM mode, the molecular ion $[M^{+}\text{-H}]$ ($mw = 325$) was fragmented within the MS to its daughter-ions ($325 > 310$ and $325 > 281$) with the cone voltage – 30 V, collision energy – 30 eV, and dwell – 0.2 sec. Argon was used as a collision gas. The daughter-ion ($mw = 281$) was used for the quantification of sterigmatocystin.

Results comparing method

For comparing of the acquired study results, Microsoft office Excel 2003 statistical functions ('t-test: Paired two sample for means' and 't-test: Two-Sample Assuming Equal Variances') were applied.

Results and Discussion

Elution of STC from two columns starts already at 45% of acetonitrile and completely elutes from the column with pure acetonitrile.

Summary results for STC eluting from the Strata X and Strata C18 SPE columns using acetonitrile and acetonitrile-water solutions are shown in Figure 2.

Using methanol-water solutions, STC elution from the column starts at 45% of methanol content in eluting solution and completely elutes from the column with pure methanol.

The results for STC elution from the Strata X and Strata C18 SPE columns using methanol and methanol-water solutions are shown in Figure 3. For comparing of STC elution procedure efficiency from Strata C18 SPE column, the 't-test: Paired two sample for means' was applied. Test results showed there were no differences between using acetonitrile and acetonitrile-water solutions and between using methanol and methanol-water solutions for elution of STC from Strata C18 column.

For comparing of STC elution procedure efficiency from Strata X SPE column, the 't-test: Paired two sample for means' was applied. Test

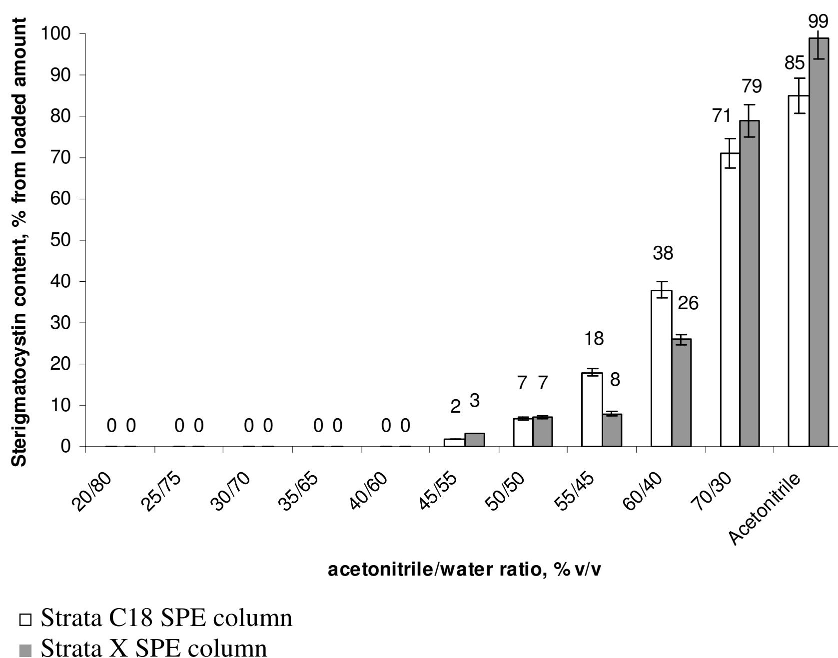

Figure 2. STC content in different acetonitrile-water fractions on Strata X and C18 SPE columns.

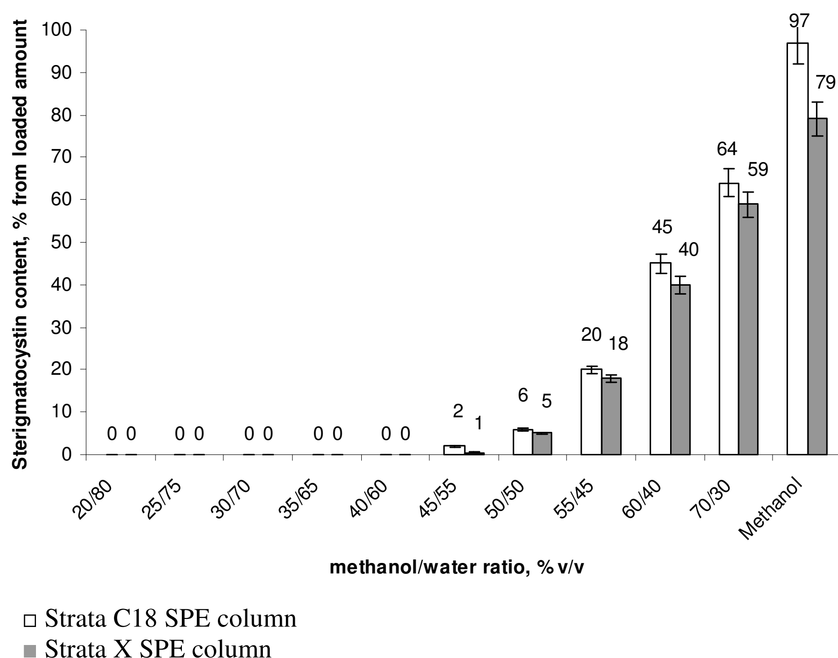

Figure 3. STC content in different methanol-water fractions on Strata X and C18 SPE columns.

results showed there were no differences between using acetonitrile and acetonitrile-water solutions and between using methanol and methanol-water solutions for elution of STC from Strata X SPE column.

For comparing of STC elution procedure efficiency from Strata C18 SPE column and from Strata X SPE column using acetonitrile and acetonitrile-water solutions the 't-test: Paired two sample for means' was applied. Test results showed there were no differences between Strata C18 SPE column and Strata X SPE column using acetonitrile and acetonitrile-water solutions.

For comparing of STC elution procedure efficiency from Strata C18 SPE column and from Strata X SPE column using methanol and methanol-water solutions, the 't-test: Paired two sample for means' was applied. Test results showed there were no differences between Strata C18 SPE column and Strata X SPE column using methanol and methanol-water solutions.

For comparing of STC elution procedures efficiency from two different SPE columns (Strata C18 SPE column and Strata X SPE column) using two different procedures (elution by acetonitrile and acetonitrile-water solutions from Strata C18 SPE column and elution by methanol and methanol-water solutions from Strata X SPE column), the 't-test: Two-Sample Assuming Equal Variances' was applied. Test results showed that there are no differences between Strata C18 SPE column and Strata X SPE column using two different elution procedures.

For comparing of STC elution procedures efficiency from two different SPE columns (Strata X SPE column and Strata C18 SPE column) using two different procedures (elution by acetonitrile and acetonitrile-water solutions from Strata X SPE column and elution by methanol and methanol-water solutions from Strata C18 SPE column), the 't-test: Two-Sample Assuming Equal Variances' was applied. Test results showed there were no differences between Strata X SPE column and Strata C18 SPE column using two different elution procedures.

Conclusions

Acetonitrile and acetonitrile-water solutions and methanol and methanol-water solutions can be applied for elution of STC from Strata X SPE column as well as from Strata C18 SPE column.

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