AN OVERVIEW ON THE CHROMATOGRAPHIC METHODS APPLIED FOR STERIGMATOCYSTIN DETECTION

Izmailova Nataliy
candidate of veterinary sciences, associate professor of the department of biochemistry and biotechnology Sumy National Agrarian University,
Chernyavska Tetyna
candidate of agricultural sciences, Associate Professor of the Department of Biochemistry and Biotechnology Sumy National Agrarian University
Ukraine

Mycotoxins are feared food contaminants, which have a negative impact on public health, food security and safety and the economy in many countries, particularly in developing ones. Some mycotoxins exhibit genotoxic, mutagenic, immunosuppressive, carcinogenic and teratogenic effects. To prevent health hazard in humans and animals regular monitoring and control of feed and food occur in almost all countries.

The mycotoxin sterigmatocystin (STC) is a precursor in the biosynthesis of aflatoxin B_1 (AFB_1). The mechanism of molecular action of STC is very similar to AFB_1. According to the results of modern research, STC was assigned to the group of the most powerful mutagens. This indicates the need to control this mycotoxin in food and feed.

Routine analysis of aflatoxins is preferably done by HPLC-FLD in all sectors dealing with food and feed. Therefore the development of a method for the simultaneous and sensitive determination of STC and AFBG would be very beneficial. The primary aim of this investigation was to develop a combined method for STC and AFBG detection.

A lot of different methods for STC detection have been performed during last decades. The methods can be divided into two groups due to their mechanism: immunochemical and chromatographic.

The basic principle of chromatographic methods is physical separation based on distribution of molecules and substances between a mobile and stationary phase. Usually these methods are applied after the sample extraction and the extract clean up. Chromatographic methods are widely used for STC detection.

In the following a summarization of chromatographic methods applied for STC detection:

Thin layer chromatography (TLC) with fluorescence detection is the most frequently used method for STC detection. Due to weak native fluorescence of STC, different methods for enhancement of the fluorescence light have been used. A validated official method includes spraying the plates with $AlCl_3$ after the development. The described procedure is conducted in order to build an STC-derivate and to change the fluorescence from red to bright yellow. The limit of quantification (LOQ) of this method is 100 μg/kg [1].

Many authors report the utilization of TLC technics with fluorescence detection for STC determination. The limits of detection (LOD) range between 20 and 50 μg/kg. The TLC method with the lowest LOD has to be mentioned: TLC with reagent-free derivitisation was reported by Stroka et al. STC was extracted from the food matrix and purified by phenyl-bond solid-phase extraction. Further heat treatment resulted in highly fluorescent spots of STC. LOD is 2-11 μg/kg and recovery about 80% [3].

Another method for quantitative analyses of STC is reverse phase HPLC. It showed absolute limit of detection 4 ng of STC [8].

In addition a reversed phase HPLC with post-column fluorescence enhancement has been developed. A post-column fluorescence enhancement is achieved through STC reaction with aluminium chloride in a solution that gives a fluorescent product: 1,3,8-trihydroxyxanthone. An interesting interrelation had been observed between mobile phase composition and fluorescence intensity. The bigger the water fraction in mobile phase composition was, the weaker was the detected fluorescence signal. If the mobile phase consisted only of methanol, the

observed fluorescence signal was 1.0. If mobile phase was 50:50 methanol-water, the fluorescence strength was 0.63. LOD was reported to be 90 μg/kg [4].

The determination of STC in feed by high-performance liquid chromatography with column switching was reported. The detection of STC was performed by UV absorption measurement at 325 nm. LOD was 25 μg/kg [9].

Using HPLC a multi-target method for the determination of patulin, penicillic acid, STC and zearalenone in samples of cocoa beans was developed. This method allowed measuring four toxins at a time. LOD for STC is 13 μg/kg [10].

One HPLC method with UV detection was applied on a beer matrix. Detection and quantification was done at 245nm and 325 nm, respectively. Reported LOD was 0.26 μg/l and LOQ was 0.68 μg/l [7].

Gas liquid chromatography tandem mass spectrometry was also used for STC detection. Limits of detection in such methods are: LOD=5 μg/kg and LOD=10 μg/kg [11].

A sensitive LC–electrospray positive ionization (ESI+) MS/MS method was developed by Versilovskis et al. The method was validated on a wheat matrix with the lowest level of 0.5 μg/kg. The LOD and LOQ of STC, determined by using standards prepared in the blank matrix, were 0.15 and 0.30 μg/kg, respectively. The high recovery of this method (97%) has to be mentioned [5] .This method was modified and applied to analyse of STC in different cheese samples by the same author. The LOD and LOQ in this study were 0.03 and 0.1 μg/kg [6].

Using the same technic, liquid chromatography/electrospray ionization tandem mass spectrometry (HPLC/ ESI-MS/MS), a method for the determination of 87 mycotoxins was developed. STC can also be measured with this method. LOD is reported to be 0.4 μg/kg [12].

A good and comprehensive overview on existing methods for STC detection was done by A. Versilovskis and S. De Saeger in 2010 [2]. The following table was directly taken from this publication and the references numbers in the right column refer to original publication.

Table 1.

Summary of chromatographic methods for the detection of STC in foodstuffs, feed, dust, building materials and indoor air

Matrix	Extraction	Defatting and clean up	Separation and detection	LOD (μg/kg)	Recovery (%)	References
Cheese	100 mL 5% NaCl + 200 mL MeOH/ acetone (50/ 50 v/v)	Liquid–liquid extraction with $CHCl_3$ and chloroform–ethyl acetate mixture, SPE on *silicagel* column	TLC-UV with $AlCl_3$	20	87	[108]
Cheese	$CHCl_3$	–	TLC-UV with $AlCl_3$	–	89–97	[109]
Cheese	MeOH/4% KCl (90/10 v/v)	SPE on *Florisil* and *polyamide* columns	TLC-UV with TFA	5	30–80	[23, 97, 110–113]
Cheese	ACN/4% KCl (85/15 v/v)	Cleanup: liquid–liquid extraction with $CHCl_3$ and cupric carbonate column	TLC-UV with Al_2Cl_6	2	86–88	[114, 115]
Cheese	$CHCl_3$	Defatting with *n*-hexane, cleanup: liquid–liquid extraction with $CHCl_3$ and then with ACN	TLC-UV with $AlCl_3$	10	–	[100]
Cheese	ACN/4% KCl (85/15 v/v)	Defatting with *n*-hexane, cleanup: liquid–liquid extraction with $CHCl_3$ and cupric carbonate diatomaceous earth column	TLC-UV	–	85–91	[24]
Cheese	ACN/H_2O (90/ 10 v/v)	Defatting with *n*-hexane. Cleanup: SPE on Strata X SPE column, then evaporation under nitrogen stream, dry residue redissolving in mobile phase	LC-ESI^+-MS/MS	0.03	96–104	[104]
Grains	MeOH/4% KCl (90/10 v/v)	Cleanup: liquid–liquid extraction with $CHCl_3$	TLC-UV with $AlCl_3$	100	60	[67]
Bread, cured ham, salami	ACN/4% KCl (90/10 v/v)	Defatting with *n*-hexane. Cleanup: liquid–liquid extraction with $CHCl_3$, SPE on *silicagel* column	TLC-UV	20	–	[72]
Grains	ACN/H_2O (90/ 10 v/v)	Defatting with *n*-hexane. Cleanup: liquid–liquid extraction with $CHCl_3$, gel permeation chromatography with *polystyrene*	GLC-MS	5	>90	[116]
Rice	Ethylacetate	Evaporation, dissolving in MeOH-20%KCl (4/1 v/v), defatting with *n*-hexane, liquid–liquid extraction with $CHCl_3$, evaporation, dry residue dissolving in acetone, SPE on Sephadex LH-20 column. Evaporation of eluate and dry residue redissolving in acetone	GLC	50	–	[89]
Bread	$CHCl_3$	Evaporation of extract, dry residue re-dissolving in $CHCl_3$ (concentration for five times)	TLC-UV with $AlCl_3$	20	–	[73]
Corn, oats, wheat	ACN/4%KCl (90/10 v/v)	Defatting with *n*-hexane, cleanup: gel permeation chromatography on *silicagel* column	TLC-UV with $AlCl_3$	30	59–138	[117]
Grains, soybeans	MeOH/4%KCl (90/10 v/v)	SPE on *Florisil* column	TLC-UV	50	92–134	[88]
Pistachio nuts	ACN/4% KCl (90/10 v/v)	Defatting with *n*-hexane. Cleanup: liquid–liquid extraction with $CHCl_3$, SPE on *silicagel* column	HPLC-UV	–	–	[91]
Corn, oats	ACN/H_2O (90/ 10 v/v)	Defatting with *n*-hexane, cleanup: gel permeation chromatography on *silicagel* column	HPLC-UV	20	59–74	[118]
Grains, corn, soybeans, feed	ACN/4% KCl (90/10 v/v)	Defatting with isooctane. Cleanup: liquid–liquid extraction with $CHCl_3$	TLC-UV with $AlCl_3$	140	92–95	[119]

Matrix	Extraction	Defatting and clean up	Separation and detection	LOD (μg/kg)	Recovery (%)	References
Maize	MeOH/$CHCl_3$, (50/50 v/v)	Defatting with *n*-hexane. Cleanup by $CHCl_3$-H_2O, and liquid–liquid extraction with $CHCl_3$	TLC-UV with $AlCl_3$	10	–	[120]
Vegetables	Ethylacetate	Defatting with *n*-hexane. Cleanup: liquid–liquid extraction with $CHCl_3$, SPE on *silicagel* column	TLC-UV with TFA anhydride	20	–	[121]
Cocoa beans	ACN/H_3PO_4 (95/5 v/v)	Defatting with *n*-hexane, cleanup: liquid–liquid extraction with $CHCl_3$ silica *Bond-elut* SPE column	HPLC-UV on CN column	13	100–108	[89]
Barley	ACN/4% KCl (90/10 v/v)	Defatting with *n*-hexane, cleanup: liquid–liquid extraction with $CHCl_3$, SPE on *silicagel* column	HPLC-FLD (precolumn derivatization with pyridine and acetic anhydride)	20	31–96	[122]
Corn, cassava flour, rice, dried black beans	MeOH/4%KCl (90/10 v/v)	Cleanup with clarifying agent and *Hyflo Super-Cel* followed by 2 partitions to $CHCl_3$	HPLC-FLD	15–35	98–117	[19]
Maize, bread, cheese	ACN/4% KCl (90/10 v/v) and MeOH/ 4%KCl (90/ 10 v/v)	Defatting with *n*-hexane, cleanup: liquid–liquid extraction with $CHCl_3$	HPLC-$APCI^+$-MS	1.7	118	[83]
				1.9	96	
				2.4	55	
Grains, corn and corn based products etc.	ACN/4% KCl (90/10 v/v)	Defatting with *n*-hexane, cleanup: liquid–liquid extraction with $CHCl_3$, SPE on s*ilicagel* column	HPLC-FLD	3.0	70–110	[93]
Grains, flour, rice	ACN/4% KCl (95/5 v/v)	Defatting with *n*-hexane, cleanup: liquid–liquid extraction with $CHCl_3$, SPE on *phenyl* column	HP-TLC	2.0	80	[60]
				2.0		
				4.0		
Rice	ACN/H_2O (85/15 v/v)	Cleanup: *MycoSep ♯226* column	GC-MS	10[a)]	72	[123]
			LC-MS/MS	4[a)]		
			HPLC-UV	2[a)]		
Grains	ACN/H_2O (84/16 v/v)	SPE on Strata X SPE column, then evaporation under nitrogen and dry residue redissolving in mobile phase	LC-ESI^+-MS/MS	0.15	80–107	[84, 124]
Bread, nuts, rice, cheese, garlic, tomato, apple, lemon, red wine, jam	ACN/H_2O/ CH_3COOH (79/20/1 v/v)	–	LC-ESI-MS/MS	0.4	101–109	[125]
Maize, peanut, pistachio, wheat, raisins, figs	ACN/H_2O (80/20 v/v)	–	LC-MS/MS	10	101–109	[126]
Beer	–	SPE on Strata X SPE column, then concentration of the dry residue and redissolving in mobile phase	HPLC-UV	0.26	81–126	[85]
Feed	$CHCl_3$/4% KCl	Concentration of the chloroform residue	TLC-UV	30	–	[127]
Feed	$CHCl_3$/H_3PO_4 (90/10 v/v)	Gel filtration	TLC-UV	50	–	[128]
Feed	MeOH/4% KCl, 90/10 v/v	Cleanup: SPE on *Florisil* column	TLC-UV with $AlCl_3$	50	92–134	[46]

Matrix	Extraction	Defatting and clean up	Separation and detection	LOD (μg/kg)	Recovery (%)	References
Different foodstuffs	ACN/4% KCl + HCl, 90/10 v/v	Defatting with *n*-hexane, cleanup: liquid–liquid extraction with CH_2Cl_2	TLC-UV	20	80	[129]
Feed, corn, silage	MeOH/4% KCl, 90/10 v/v	Cleanup: SPE on *Florisil* column	TLC-UV with $AlCl_3$	2	–	[94]
Silage	$CHCl_3$	Cleanup: liquid–liquid extraction with H_2O and $CHCl_3$	TLC-UV with $AlCl_3$	–	–	[130]
	ACN/H_2O	–	HPLC-UV	–	–	[62]
	MeOH	–	HPLC-UV	–	–	[63]
Building materials, indoor air, dust	ACN	Filtering through glass fiber filter, dry residue redissolving in MeOH/H_2O (50/50 v/v)	LC-ESI^+-MS/MS	2–4	33	[61]
	Ethyl acetate, then with CH_2Cl_2	Filtering through folded filter, dry residue redissolving in MeOH/H_2O (30/70 v/v)	LC-ESI^+-MS/MS	1.1–1.5 μg/L	61	[64]
	ACN/H_2O + H_3PO_4 (pH 1.5)	Cleanup: SPE on *Chromabond XTR* column	HPLC-UV	100	50–64	[131]

from [2]

Conclusion. The article mentions a number of experiments in which the mutagenicity and carcinogenic potential of a given chemical substance was evaluated. According to their results, STC was assigned to the group of the most powerful mutagens.

All this indicates the need to monitor this mycotoxin in food and feed.). Especially the occurrence of STC at low levels remains questionable, due to the high limit of detection of most methods used for its determination. Features of solubility and other physical and chemical properties of STC does not permit the application of existing methods for determining mycotoxins to it. Therefore, it is important to develop a sensitive method for determining STC along with other mycotoxins.

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