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Research Article

A basic tool for risk assessment: A new method for the analysis of ergot alkaloids in rye and selected rye products

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As a basis for the collection of occurrence and exposure data of ergot alkaloids in food, an HPLC method coupled with fluorimetric detection (HPLC-FLD) for the determination of 12 pharmacologically active ergot alkaloids in rye and rye products was developed. Samples were extracted with a mixture of ethyl acetate, methanol, and aqueous ammonia, followed by centrifugation and purification by solid phase filtration (SPF) with basic alumina. After solvent adjustment, the samples were analyzed by HPLC-FLD using a phenyl–hexyl-column. Recoveries for five major alkaloids were between 89.3% (ergotamine) and 99.8% (α-ergokryptine) with a maximum LOQ of 3.3 μg/kg (ergometrine). Precision expressed as RSD ranged from 2.8% (ergocristine) to 12.4% (α-ergokryptine) for repeatability, and from 6.5% (ergocornine) to 14.9% (ergotamine) for within-laboratory reproducibility, respectively. In a survey of 39 rye product samples, ergocristine and ergotamine were found to be the major alkaloids in commercially available rye products with contents of 127 μg/kg (ergocristine), and 134 μg/kg (ergotamine) in rye flour, and 152.5 and 117.8 μg/kg in coarse meal, respectively.

Keywords: *Claviceps* / Ergot alkaloids / HPLC-FLD / Rye / Validation

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1 Introduction

Ergot alkaloids are mainly produced by the fastidious parasitic fungus *Claviceps* spp., infecting the ovaries of various *Poaceae* such as rye, wheat, triticale, barley, millet, and grasses of which rye, triticale and grasses are the most affected ones [1–5].

These violet to black colored ergots or sclerotia (*Secale cornutum*) that are formed instead of sane grains contain 0.1–0.8% of pharmacologically active indole alkaloids, exerting toxic effects on endotherms [5–8]. Known as St. Anthony's fire, ergot containing cereals caused numerous epidemics of acute, gangrenous ergotism in the middle ages [9–11]. The observed α-adrenoceptor-mediated vasoconstriction is characterized by swelling, reddening, and, in the last resort, by necrosis, loss of extremities, and death [7, 9, 12]. Chronic ergot alkaloid intake impacts the central nervous system inducing giddiness, formication, nausea, flexion of extremities, paralysis, psychosis, dementia, and death [7–10].

Acute intoxication manifests symptoms of nausea, headache, paraesthesia, convulsion, death by respiratory paralysis, and circulatory collapse while doses of 5–10 g can be lethal [6, 7]. Pregnant women may come down with uterus contraction in association with bleeding and tetanus uteri followed by suffocation of the embryo, miscarriage, and uterus rupture [8, 9].

All ergot alkaloids have the indole containing tetracyclic ergoline system in common (Fig. 1). They are derivatives of D-lysergic acid (suffix "-ine") and D-iso-lysergic acid (suffix "-inine") that can isomerize into one another at which D-iso-lysergic acid shows less pharmacological activity on endotherms [5].

For the determination of ergot alkaloids in products of plant origin, a variety of analytical techniques such as colorimetric analysis with van Urk's reagent [2–4], thin layer chromatography [13–15], immunoassays [16–18], CZE [19, 20], HPLC coupled with fluorimetric detection (HPLC-FLD) [6, 21–28], and LC coupled with MS/MS [26, 29–32] has been reported.

Due to decomposition in the injector system, GC is only used for the identification of the peptide moiety, especially

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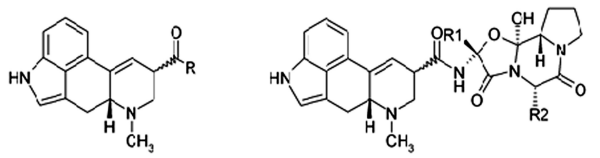

A - Alkanolamides B - Ergopeptides

Compound	Type	Moiety R	Moiety R1	Moiety R2
Lysergic acid diethyl amide (LSD)	Alkanolamide	NH $(C_2H_5)_2$	—	—
Ergometrine/ Ergometrinine	Alkanolamide	NH CH (CH_3) CH_2 OH	—	—
Ergocornine/ Ergocorninine	Ergopeptide	—	CH $(CH_3)_2$	CH $(CH_3)_2$
Ergocristine/ Ergocristinine	Ergopeptide	—	CH $(CH_3)_2$	CH_2 C_6H_5
Ergokryptine/ Ergokryptinine	Ergopeptide	—	CH $(CH_3)_2$	CH_2 CH $(CH_3)_2$
Ergosine/ Ergosinine	Ergopeptide	—	CH_3	CH_2 CH $(CH_3)_2$
Ergotamine/ Ergotaminine	Ergopeptide	—	CH_3	CH_2 C_6H_5

Figure 1. Molecular structure of 13 most prominent ergot alkaloids and related compounds.

for pharmaceutical and forensic issues [33]. HPLC-FLD analysis is the most commonly applied method due to fluorescence sensitivity of the conjugated ergoline system (Fig. 1).

At present, there is no method on hand that is validated according to approved protocols such as ISO/CEN, DIN, or German Food and Feed Code, Section 64 (Lebensmittel- und Futtermittelgesetzbuch – LFGB, § 64). Hence, only sparse occurrence data of ergot alkaloids in human nutrition, and exposure data for humans as a basis for risk assessment, are available [34]. So, it was the aim of this work to provide an efficient, thoroughly in-house validated method, applicable with only little effort concerning time, cleanup, solvent demand, and detection system, and without the use of halogenated solvents as required for German Food Monitoring. Collected occurrence data will be the basis for the risk assessment of ergot alkaloids in food.

2 Materials and methods

2.1 Chemicals, reagents, and materials

Ergocornine, ergocorninine, ergocristine, ergocristinine, α-ergokryptine, α-ergokryptinine, ergometrine maleate, ergometrinine, ergosine, ergosinine, ergotamine tartrate, and ergotaminine were kindly provided as thin films by the Federal Office of Public Health (Bern, Switzerland).

Spiking standard substances (ergocornine, ergocristine, α-ergokryptine, ergometrine maleate, and ergotamine tartrate) for recovery experiments were obtained from Sigma – Aldrich (Steinheim, Germany).

Solvents and chemicals used were all of analytical grade or higher purity standard. ACN, ethyl acetate, and ammonium carbamate were from Merck (Darmstadt, Germany). Methanol and aqueous ammonia solution (25%) were purchased from Fluka (Buchs, Switzerland).

SepPack® Alumina B cartridges (sorbent weight 1710 mg, particle size 50 – 300 μm) were from Waters (Eschborn, Germany).

2.2 Calibration solutions

Stock solutions were prepared by redissolving the thin film standard compounds in the mobile phase (ACN – ammonium carbamate buffer (0.2 g/L) (50:50 v/v)), and were adjusted to a concentration of 250 ng/mL *per* compound. Calibrants were prepared by diluting the stock solution with the same solvent mixture in a range from 1 to 50 ng/mL corresponding to an analyte concentration of 5 – 250 μg/kg sample. Amber glassware was used to prevent from photodegradation and isomerization of the ergot alkaloids. Stock solutions and diluted standard solutions were stored at –30°C.

2.3 Apparatus and analytical conditions

An Agilent 1100 Series system (Agilent Technologies, Waldbronn, Germany) consisting of a membrane degasser, a binary pump, a thermostated autosampler with a 100 μL loop, a thermostated column compartment, and a fluorescence detector set at 315 nm (excitation) and 415 nm (emission) was used for analyses. The column was a Gemini C6-phenyl, 250 mm × 4.6 mm, particle size 5 μm, purchased from Phenomenex (Aschaffenburg, Germany). The mobile phase consisted of a mixture of ACN and ammonium carbamate buffer (0.2 g/L) (50:50 v/v). The column temperature was set at 30°C.

Using isocratic elution, the following flow program was applied: 0 – 16 min at 0.8 mL/min, 16 – 29 min at 1.5 mL/min, and 29 – 32 min at 0.8 mL/min.

Reproducibility tests were performed on a Shimadzu (Duisburg, Germany) 10-A system with a membrane degasser, a binary pump, a thermostated autosampler with a 500 μL loop, a thermostated column compartment, and a fluorescence detector. The same analytical conditions as mentioned above were applied.

2.4 Samples

Rye and rye products (flour, flakes, and coarse wholemeal) samples were purchased from local German supermarkets, health food stores, and flour mills. Cereals, flakes, and coarse wholemeal samples were ground to a particle size of 0.5 mm using a Retsch ZM 100 mill (Haan, Germany). The flour was homogenized by thorough shaking. The samples were stored in darkness at room temperature until analysis.

2.5 Method development

Method development was based on the extraction solvent introduced by Scott and Lawrence [28], which consists of a mixture of dichloromethane, ethyl acetate, methanol, and

ammonia hydroxide solution (25%) (50:25:5:1 by volume). To avoid halogenated solvents, the ethyl acetate content was raised to 75 parts, entailing the necessity to adjust the solvent polarity by adding 7 parts of ammonia hydroxide solution (25%) in order to enhance ergometrine and ergometrinine extraction efficiency, respectively.

2.6 Sample preparation

Twenty grams of the sample were extracted in 250 mL polypropylene bottles with 100 mL of a mixture of ethyl acetate–methanol–ammonia hydroxide solution (25%) (75:5:7 by volume) by turbulent shaking for 45 min (Turbula T2C and F, Willy A. Bachofen, Basel Switzerland). The extraction solvent was shaken thoroughly before each removal of an aliquot. After centrifugation (Beckmann, J2-21, Krefeld, Germany) at 10°C and 5000 rpm for 20 min, 5 mL of the raw extract were transferred on a basic alumina cartridge (Sep-Pak® Plus Alumina B cartridge sorbent weight 1710 mg, particle size 50–300 μm, Waters). An aliquot of the eluate (2.0 mL) was evaporated to dryness in the nitrogen stream at 45°C and approximately 0.34 bar (Zymark TurboVap LV, Hopkinton, MA, USA), redissolved in 2.0 mL of the HPLC eluent by means of ultrasonic bath (Transsonic T460, Elma, Singen, Germany) for 20 min and centrifuged (Beckman Microfuge R, Krefeld, Germany) at 10°C and 15 300 rpm for 10 min using ultrafree-MC 0.45 μm centrifugal filters (Millipore, Billerica, MA, USA). Purified filtrate (10 μL) was analyzed by HPLC-FLD.

3 Results and discussion

3.1 Liquid chromatographic separation

Separation of the 12 ergot alkaloids was performed within 32 min under isocratic conditions. Figure 2 shows a chromatogram of a calibrant (I) containing approximately 5 ng/mL (corresponding to 25 μg alkaloid *per* kg sample) *per* compound and a naturally contaminated rye flour (II).

3.2 Linearity

The linearity of the calibration function was verified by a Mandel test of goodness of fit [35] within a range of approximately 0.01–0.60 ng/Inj (5–300 μg/kg, Table 1) *per* compound. In addition, analysis of residual plots did not show any irregular pattern.

3.3 LOD and LOQ

LOD and LOQ were estimated according to German Standard, DIN 32645 [36], by analyzing a ground rye sample with ten replicates. An α-error of 1% and a confidence range of ±33.3% ($k = 3$) were applied.

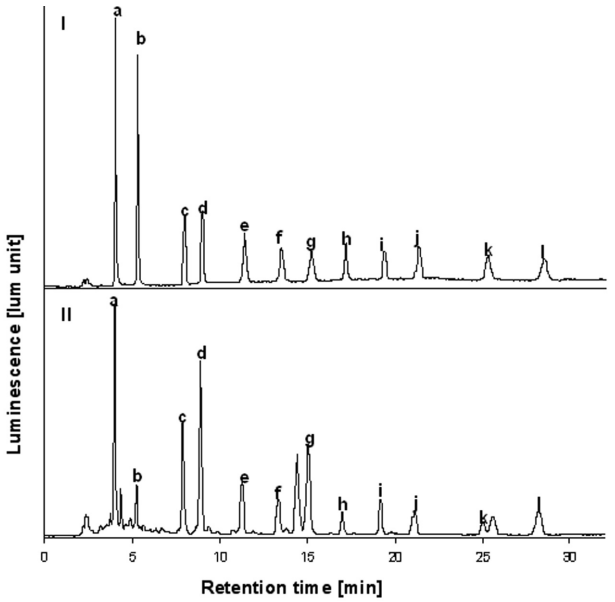

Figure 2. Chromatogram of an HPLC calibrant (I) containing approximately 25 μg/kg *per* compound and naturally contaminated rye flour (II); (a) ergometrine (55 μg/kg), (b) ergometrinine (16 μg/kg), (c) ergosine (81 μg/kg), (d) ergotamine (133 μg/kg), (e) ergocornine (52 μg/kg), (f) α-ergokryptine (47 μg/kg), (g) ergocristine (127 μg/kg), (h) ergosinine (31 μg/kg), (i) ergotaminine (54 μg/kg), (j) ergocorninine (34 μg/kg), (k) α-ergokryptinine (27 μg/kg), (l) ergocristinine (59 μg/kg).

Blank method results ranged from 0.02 (ergocristine) to 1.10 μg/kg (ergometrine) for the LOD, and from 0.08 to 3.30 μg/kg for the LOQ, respectively (Table 2). These values are low and comparable with previously published HPLC-FLD methods (LOD: 0.6–4.0 μg/kg [6]; 0.2–1.1 μg/kg [37]; LOQ: 4 μg/kg [23]; 2.8–17.9 μg/kg [24]).

Mohamed *et al*. [29] reported LODs of 7 (ergometrine, ergotamine, ergocornine, ergokryptine) and 11 μg/kg (ergocristine) and LOQs of 23 and 37 μg/kg for an LC-MS/MS-based method estimated by a modified blank method.

In a second approach, LOD and LOQ were approximated by extrapolation from the recovery experiments (see Section 3.4) according to DIN 32645 calibration method [36], although the spiking level was too high and should normally range between LOD and LOQ. The same statistical parameters as mentioned above were applied.

Calibration method results ranged from 8.1 (ergometrine) to 22.3 μg/kg (ergocristine) for the LOD, and from 25.5 to 66.5 μg/kg for LOQ, respectively (Table 2).

Assessment of the LOD and LOQ suitability was difficult, since no maximum levels or comparable limits for ergot alkaloids in food are presently established in German and European legislation. So, the present approach was designed to verify the requirements resulting from the former maximum level of 0.05% for ergot sclerotia in cereals according to German Intervention Guideline [38]. Based on a mean alkaloid content of 0.2% in sclerotia [5, 7], 0.05%

Table 1. Recovery efficiencies for the analysis of ergot alkaloids in cereals and flours

	Ergo-cornine	Ergo-corninine	Ergo-cristine	Ergo-cristinine	α-Ergo-kryptine	α-Ergo-kryptinine	Ergo-metrine	Ergo-sine	Ergo-sinine	Ergo-tamine	Ergo-taminine
Tested range[a] (μg/kg)	5.3 – 266.0	5.1 – 254.0	4.7 – 234.5	5.8 – 291.0	4.7 – 235.5	5.3 – 265.0	5.4 – 271.0	6.5 – 323.5	5.4 – 269.5	5.3 – 264.0	6.2 – 309.5
Recovery[b] (%)	98.6 ± 3.8	n.a.[c]	97.6 ± 3.1	n.a.[c]	99.8 ± 2.4	n.a.[c]	99.2 ± 3.8	n.a.[c]	n.a.[c]	89,3 ± 3.4	n.a.[c]
Coefficient of determination	0.9961	n.a.[c]	0.9942	n.a.[c]	0.9967	n.a.[c]	0.9853	n.a.[c]	n.a.[c]	0.977	n.a.[c]

a) For standard solutions.
b) Recovery ± CV; within a range from approximately 25 to 130 μg/kg.
c) n.a. not analyzed.

Table 2. Comparison of estimated ergot alkaloid contents in cereals, containing 0.05% sclerotia with an alkaloid content of 0.2%, with the method's LOQ's

Compound	Percental contribution to total alkaloid content according to ref. [12] (%)	Estimated contents (μg/kg)	LOQ calculated by blank method (μg/kg)	LOQ calculated by calibration method (μg/kg)
Ergocornine	4	40	0.19	36.51
Ergocorninine	2	20	0.16	n a.[a]
Ergocristine	31	310	0.08	66.45
Ergocristinine	13	130	0.88	n.a.[a]
Ergokryptine	5	50	0.14	55.88
Ergokryptinine	3	30	0.09	n.a.[a]
Ergometrine	5	50	3.30	25.55
Ergometrinine	2	20	0.55	n.a.[a]
Ergosine	4	40	0.55	n.a.[a]
Ergosinine	2	20	0.18	n.a.[a]
Ergotamine	17	170	0.56	57.58
Ergotaminine	8	80	0.13	n.a.[a]

a) n.a. not analyzed.

sclerotia in cereals correspond to a total mean alkaloid content of 1000 μg/kg. In addition, EFSA [12] reported a mean alkaloid distribution as listed in Table 2, so that contents for each alkaloid could be estimated.

The demonstrated LOQs meet the requirements for quantitation of naturally occurring ergot alkaloid contents in cereals and flours with one exception: α-ergokryptine estimated by the calibration method.

Since the conservative calibration method provides only additional assessment criteria, and the estimated content is an approximation, the method for the analysis of ergot alkaloids in cereals is considered to be sufficient.

In the following, LOQs deriving from blank method are used for further assessment of the analyzed samples (see Section 3.6), since DIN 32645 [36] states that this method shall be authoritative, and LOQs according to the calibration method could only be determined for five out of a total of twelve ergot alkaloids.

3.4 Recovery efficiencies

Recovery efficiencies were checked for five ergot alkaloids (ergocornine, ergocristine, α-ergokryptine, ergometrine, and ergotamine), selected as being representative, by spiking blank material (ground rye). For the other compounds, no reference standards were available in the required amounts necessary for the spiking procedure. Tested concentrations ranged between 25 and 150 μg/kg in four equidistant steps and four replicates each.

Recoveries were linear functions of the concentration, verified by a residual analysis and a Mandel test of goodness of fit [35], and ranged between 89.3 (ergotamine) and 99.8% (α-ergokryptine).

These results are similar and good compared with recoveries of previously published methods of 61 – 97% [6], 74 – 82% [23], 79 – 96% [24], and 58 – 65% [37].

Recovery efficiencies ± CVs, and related coefficients of determination (r^2) are listed in Table 1.

3.5 Method precision

Precision was proved by repeatability (RSD_r) and within-laboratory reproducibility (RSD_R).

Naturally contaminated rye flour was analyzed nine times by one operator on 1 day for repeatability studies. In-house reproducibility studies were conducted with the same material by two operators (one trained, one untrained) on 6 days with three replicates corresponding to a total number

Table 3. Precision data for the analysis of ergot alkaloids in rye flour

	Alkaloid content (μg/kg)										
	Ergo-cornine	Ergo-corninine	Ergo-cristine	Ergo-cristinine	α-Ergo-kryptine	α-Ergo-kryptinine	Ergo-metrine	Ergo-sine	Ergo-sinine	Ergo-tamine	Ergo-taminine
Σ302.6	22.4	15.2	55.9	25.2	20.5	12.4	22.9	30.9	14.6	57.3	25.4
Under repeatability conditions (n = 9)											
SD (μg/kg)	1.1	1.0	1.5	1.0	1.1	1.4	1.8	1.6	0.6	2.6	1.5
CV (%)	4.8	6.5	2.8	4.6	5.6	12.4	7.4	5.1	4.4	5.5	6.5
Under within-laboratory reproducibility conditions (n = 18)											
SD (μg/kg)	1.9	1.0	3.9	2.5	2.3	0.9	1.7	3.0	1.8	6.1	3.9
CV (%)	8.4	6.5	6.8	9.6	10.9	6.7	7.7	9.8	12.1	9.8	14.9

of 18 samples. All data were checked for normal distribution (R/s- and David test), outliers (Grubbs test), and trends (Neumann test) with an α-error of 1%.

To our knowledge, precision for an ergot alkaloid HPLC-FLD method was not studied to such an extend with characterization of both repeatability and within laboratory reproducibility. Ware *et al*. [24] reported repeatability CVs deriving from recovery experiments ($n = 6$ replicates) ranging from 1.6 to 15.4%. Storm *et al*. presented similar results ($n = 8$ replicates) ranging between 8.4 and 13.0%.

Mohamed *et al*. [29] determined precision with 3–9 mg/kg *per* alkaloid spiked material for their in-house validated LC-MS/MS method. Results ranged from 8 to 17% for both repeatability and within-laboratory reproducibility for five major ergot alkaloids.

The here presented method is characterized by CV's ranging from 3 to 12% under repeatability conditions, and from 7 to 15% under within-reproducibility conditions for a naturally contaminated flour with a total alkaloid content of 302 μg/kg *per* 11 alkaloids. Results are depicted in Table 3.

Compliance levels for precision RSDs in ergot alkaloid analysis are not available. Hence, the obtained data were compared with further criteria to evaluate compliance. In the case of products of animal origin, the Commission Decision 2002/657/EC [39] specified a maximum RSD_R of 23% according to the Horwitz equation in a mass range between 100 and 999 μg/kg. For mass ranges below 100 μg/kg, RSD_R is supposed to be as low as possible. Besides, Horwitz equation defines an RSD_r value of 15% for concentrations above 100 μg/kg [39–41].

Respective RSDs ranged from 2.8% (ergocristine) to 12.4% (α-ergokryptine) for repeatability and from 6.5% (ergocornine) to 14.9% (ergotamine) for within-laboratory reproducibility. These values are below the Decision 2002/657/EC [39] criterion of 23 and 15% respectively, according to Horwitz equation [39–41].

Obtained precision data are clearly below the target values of decision 2002/657/EC. Therefore, the developed method has been found as being suitable for ergot alkaloid trace analysis.

3.6 Application

Thirty-nine harvest products of 2006/2007 (rye and rye product samples) were investigated for their ergot alkaloid content. Mean, median, and maximum concentrations for each alkaloid as well as the total alkaloid content are shown in Table 4. The incidence of positive samples and the between-samples variability calculated as ratio of between-sample SD and overall mean are also depicted in Table 4.

In rye flour ($n = 22$), the incidence for total alkaloids was 100%. The highest concentration of ergot alkaloids was found in rye flour from a local flour mill with a total alkaloid content of 714.6 μg/kg. Ergocristine and ergotamine were the major alkaloids according to their mean (maximum) content of 27.0 (126.9) and 24.4 (132.9) μg/kg, respectively, confirming the observations by Young [2, 3], and Young and Chen [4].

These results are similar to the results of the coarse meal samples ($n = 7$). The highest total alkaloid content (739.7 μg/kg) was found in a coarse meal sample bought from a local wholefood-bakery. Again, ergocristine and ergotamine were the major alkaloids according to their mean (maximum) content of 25.6 (152.5) and 23.3 (117.8) μg/kg.

The occurrence of ergotamine was not as important in rye samples ($n = 7$) as in flour and coarse meal with a mean (maximum) content of 7.2 (20.9) μg/kg. The major alkaloid ergocristine was present in rye with a mean (maximum) concentration of 17.3 (54.3) μg/kg.

Contamination of rye flakes ($n = 3$) was very low with a maximum total alkaloid content of 66.2 μg/kg, although further samples have to be analyzed to prove significance of these results.

Between-sample variability for all samples ranged between approximately 90 and 265% *per* alkaloid which is

Table 4. Ergot alkaloid contents in rye products

	Ergo-cornine	Ergo-corninine	Ergo-cristine	Ergo-cristinine	α-Ergo-kryptine	α-Ergo-kryptinine	Ergo-metrine	Ergo-metrinine	Ergo-sine	Ergo-sinine	Ergo-tamine	Ergo-taminine	Total alkaloid content
Rye flour (n = 22)													
Mean (μg/kg)	10.7	6.2	27.0	8.7	15.8	4.2	7.9	1.8	14.3	7.4	24.4	9.3	137.5
Median (μg/kg)	6.7	3.9	14.2	1.9	12.0	0.0	6.6	0.0	8.8	5.9	10.7	5.4	85.0
Maximum (μg/kg)	52.3	34.2	126.9	58.9	58.2	26.9	53.3	15.8	81.2	31.3	132.9	54.3	714.6
Between-Sample Var. (%)	130.9	138.9	129.3	169.2	101.6	167.0	138.7	210.1	141.9	113.1	143.1	153.6	130.5
Incidence positive samples (%)	72.7	68.2	81.8	50.0	77.3	45.5	77.3	45.5	68.2	68.2	77.3	63.6	100.0
Rye (n = 7)													
Mean (μg/kg)	4.7	3.7	17.3	2.7	6.4	1.7	4.5	0.7	9.0	2.5	7.2	1.8	62.2
Median (μg/kg)	0.0	0.0	14.6	0.0	0.0	0.0	2.7	0.0	0.0	0.0	4.3	0.0	59.7
Maximum (μg/kg)	23.3	15.2	54.3	10.4	29.5	12.1	15.9	4.8	35.7	17.5	20.9	6.7	197.0
Between-sample Var. (%)	189.8	164.4	112.0	155.1	175.9	264.6	130.0	264.6	165.3	264.6	115.9	156.6	109.1
Incidence positive samples (%)	42.9	42.9	85.7	42.9	42.9	14.3	57.1	14.3	42.9	14.3	57.1	42.9	85.7
Rye coarse meal (n = 7)													
Mean (μg/kg)	18.9	10.2	25.6	10.0	18.2	6.9	11.5	0.0	15.5	8.0	23.3	9.6	157.7
Median (μg/kg)	7.2	5.9	5.0	0.0	13.0	4.4	8.4	0.0	7.7	3.7	10.6	0.0	90.5
Maximum (μg/kg)	88.0	46.0	152.5	55.7	64.2	31.3	30.2	0.0	69.5	32.1	117.8	52.4	739.7
Between-sample var. (%)	166.5	158.1	219.0	204.6	120.0	162.2	83.2	–	159.4	146.2	164.8	180.3	164.7
Incidence positive samples (%)	71.4	71.4	71.4	42.9	71.4	57.1	85.7	0.0	71.4	57.1	85.7	42.9	85.7
Rye flakes (n = 3)													
Mean (μg/kg)	5.5	3.7	4.9	0.0	3.8	1.7	4.3	0.0	0.0	0.0	2.4	0.0	26.4
Median (μg/kg)	0.0	0.0	0.0	0.0	0.0	0.0	4.3	0.0	0.0	0.0	2.9	0.0	12.9
Maximum (μg/kg)	16.6	11.2	14.6	0.0	11.5	5.1	8.6	0.0	0.0	0.0	4.3	0.0	66.2
Between-sample var. (%)	173.2	173.2	173.2	–	173.2	173.2	100.0	–	–	–	91.4	–	133.1
Incidence positive samples (%)	33.3	33.3	33.3	0.0	33.3	33.3	66.7	0.0	0.0	0.0	66.7	0.0	66.7

not unusual. Young [2] reported a variation of the total alkaloid content of more than 4000% (total alkaloid content between 0.011 and 0.452%) in rye sclerotia of one field.

As mentioned above, the former maximum level of 0.05% for ergot sclerotia in cereals according to German Intervention Guideline [38] corresponds to a total mean alkaloid content of 1000 μg/kg. This value is not exceeded by any of the analyzed samples.

Scott *et al*. [27] reported mean total alkaloid contents in rye flour calculated for six major alkaloids (ergocornine, ergocristine, ergokryptine, ergometrine, ergosine, and ergotamine) for the harvests 1985/1986 – 1990/1991, ranging between 69 (1985/1986) and 414 μg/kg (1986/1987) with ergocristine and ergotamine being the most prominent alkaloids.

Although generally lower, the obtained alkaloid contents are in the line with the data collected by Scott *et al*. [27].

More recently, Lauber *et al*. [22] published mean alkaloid contents in rye flours and grains calculated for 12 alkaloids amounting to 818 μg/kg in 2003, and 260 μg/kg in 2004, respectively. In 2003, 23% of the analyzed samples exceeded the former maximum level of 1000 μg/kg, one with a maximum total alkaloid content of 3280 μg/kg.

The obtained data are in a line with the results of the crop year 2004, whereas data of the year 2003 cannot be confirmed. The explanation given in ref. [22] relates to the strong influence of climatic conditions causing smaller ergot bodies under adverse circumstances. These smaller sclerotia may cause severe problems in separating them from the sane kernels.

4 Concluding remarks

An analytical method based on solid phase filtration (SPF) clean-up followed by liquid chromatographic separation on a phenyl – hexyl-column and fluorescence detection for ergot alkaloids in cereals and flours was developed and

inhouse-validated in terms of limits of detection and quantitation, recovery and precision. Acceptable recoveries could be achieved, ranging from 89.3% (ergotamine) to 99.8% (α-ergokryptine) with a maximum LOQ of 3.3 µg/kg (ergometrine) calculated by blank method and of 66.5 µg/kg (ergocristine) calculated by calibration method.

Data obtained for repeatability and inhouse-reproducibility proved the precision and robustness of the described method.

Additionally, this method was applied to 39 rye and rye product samples, all below the former maximum level of 1000 µg/kg (corresponding to a sclerotia content of 0.05% in rye). In general, the obtained data are in a line with previously published data from Canada [27] and Germany [22], although observed total alkaloid contents tend to be lower probably caused by different climatic conditions.

Due to its good manageability and successful validation for the six ergot alkaloids ergocornine, ergocristine, α-ergokryptine, ergometrine, and ergotamine, the presented method will be standardized and published in the Official Compilation of Test Methods according to German Food and Feed Code, Section 64, as a routine and reference method for the German Food Surveillance.

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5 References

[1] Hofmann, A., *Die Mutterkornalkaloide*, Nachtschatten Verlag, Solothurn 2000.

[2] Young, J. C., Variability in the content and composition of alkaloids found in Canadian ergot. I. Rye, *J. Environ. Sci. Health* 1981, *16*, 83 – 111.

[3] Young, J. C., Variability in the content and composition of alkaloids found in Canadian ergot. II. Wheat, *J. Environ. Sci. Health* 1981, *16*, 381 – 393.

[4] Young, J. C., Chen, Z., Variability in the content and composition of alkaloid found in Canadian ergot. III. Triticale and barley, *J. Environ. Sci. Health* 1982, *17*, 93 – 107.

[5] Mielke, H., *Studien über den Pilz Claviceps purpurea (Fries) Tulasne unter Berücksichtigung der Anfälligkeit verschiedener Roggensorten und der Bekämpfungsmöglichkeiten des Erregers,* Parey-Verlag, Berlin 2000.

[6] Klug, C., *Bestimmung von Mutterkornalkaloiden in Lebensmitteln*, Max von Pettenkofer-Institut des Bundesgesundheitsamtes, Berlin 1986.

[7] Guggisberg, H., *Mutterkorn – Vom Gift zum Heilstoff*, S. Karger AG, Basel 1954.

[8] Schoch, U., Schlatter, C., Gesundheitsrisiken durch Mutterkorn aus Getreide, *Mitt. Gebiete Lebensm. Hyg.* 1985, *76*, 631 – 644.

[9] van Dongen, P. W. J., de Groot, A. N. J. A., History of ergot alkaloids from ergotism to ergometrine, *Obstet. Gynecol.* 1995, *60*, 109 – 116.

[10] Schiff, R. L., Ergot and its alkaloids, *Am. J. Pharm. Educ.* 2006, *70*, 1 – 10.

[11] Richard, J. L., Some major mycotoxins and their mycotoxicoses-An overview, *Int. J. Food. Microbiol.* 2007, *119*, 3 – 10.

[12] EFSA, Opinion of the Scientific Panel on Contaminants in Food Chain on a request from the Commission related to ergot as undesirable substance in animal feed, *EFSA J.* 2005, *225*, 1 – 27.

[13] Urga, K., Debella, A., W/Medihn, Y., Agata, N., *et al*., Laboratory studies on the outbreak of gangrenous ergotism associated with consumption of contaminated barley in Arsi, Ethiopia, *Ethiop. J. Health Dev.* 2002, *16*, 317 – 323.

[14] Salvat, A. E., Godoy, H. M., A simple thin-layer chromatographic method for the detection of ergovaline in leaf sheaths of tall fescue (Festuca arundinacea) infected with Neotyphodium coenophialum, *J. Vet. Diagn. Invest.* 2001, *13*, 446 – 449.

[15] Sallam, L. A. R., Naim, N., El-Refai, A. H., Thin-layer chromatography of some ergot alkaloids, *Z. Anal. Chem.* 1977, *284*, 47 – 48.

[16] Molloy, J. B., Moore, C. J., Bruyeres, A. G., Murray, S.-A., Blaney, B. J., Determination of dihydroergosine in sorghum ergot using immunoassay, *J. Agric. Food Chem.* 2003, *51*, 3916 – 3919.

[17] Schnitzius, J. M., Hill, N. S., Thompson, C. S., Craig, A. M., Semiquantitative determination of ergot alkaloids in seed, straw, and digesta samples using a competitive enzyme-linked immunosorbent assay, *J. Vet. Diag. Invest.* 2001, *13*, 230 – 237.

[18] Shelby, R. A., Kelley, V. C., Detection of ergot alkaloids from Claviceps species in agricultural products by competitive ELISA using a monoclonal antibody, *J. Agric. Food Chem.* 1992, *40*, 1090 – 11092.

[19] Frach, K., Blaschke, G., Separation of ergot alkaloids and their epimers and determination in sclerotia by capillary electrophoresis, *J. Chromatogr. A* 1998, *808*, 247 – 252.

[20] Fanali, S., Flieger, M., Steinerova, N., Nardi, A., Use of cyclodextrins fort he enatioselective speration of ergot alkaloids by capillary zone electrophoresis, *Electrophoresis* 1992, *13*, 39 – 43.

[21] Müller, C., Klaffke, H. S., Krauthause, W., Wittkowski, R., Determination of ergot alkaloids in rye and rye flour, *Mycotox. Res*. 2006, *22*, 197 – 200.

[22] Lauber, U., Schnaufer, R., Gredziak, M., Kiesswetter, Y., Analysis of rye grains and rye meals for ergot alkaloids, *Mycotox. Res*. 2005, *21*, 258 – 262.

[23] Lombaert, G. A., Pellaers, P., Roscoe, V., Mankotia, M., *et al*., Mycotoxins in infant cereal foods from the Canadian market. *Food Addit. Contamin.* 2003, *20*, 494 – 504.

[24] Ware, G. M., Price, G., Carter, L., Jr., Eitenmiller, R. R., Liquid chromatographic preparative method for isolating ergot alkaloids, using a particle-loaded membrane extracting disk, *J. AOAC Int.* 2000, *83*, 1395 – 1399.

[25] Fajardo, J. E., Dexter, J. E., Roscoe, M. M., Nowicki, T. W., Retention of ergot alkaloids in wheat during processing, *Cereal Chem.* 1995, *72*, 291 – 298.

[26] Rottinghaus, G. E., Schultz, L. M., Ross, F., Hill, N. S., An HPLC method for the detection of ergot in ground and pelleted feeds, *J. Vet. Diagn. Invest.* 1993, *5*, 242 – 247.

[27] Scott, P. M., Lombaert, G. A., Pellaers, P., Bacler, S., Lappi, J., Ergot alkaloids in grain foods sold in Canada, *J. AOAC Int.* 1992, *75*, 773 – 779.

[28] Scott, P. M., Lawrence, G. A., Analysis of ergot alkaloids in flour, *J. Agric Food Chem.* 1980, *28*, 1258 – 1261.

[29] Mohamed, R., Gremaud, E., Richoz-Payot, J., Tabet, J.-C., Guy, P. A., Quantitative determination of five ergot alkaloids in rye flour by liquid chromatography-electrospray ionisation tandem mass spectrometry, *J. Chromatogr. A* 2006, *1114*, 62 – 72.

[30] Bürk, G., Höbel, W., Richt, A., Ergot alkaloids in cereal products. Results from the Bavarian Health and Food Safety Authority, *Mol. Nutr. Food Res.* 2006, *50*, 437 – 442.

[31] Sulyok, M., Berthiller, F., Krska, R., Schumacher, R., Development and validation of a liquid chromatography/tandem mass spectrometric method for the determination of 39 mcotoxins in wheat and maize, *Rapid Commun. Mass Spectrom.* 2006, *20*, 2649 – 2659.

[32] Lehner, A. F., Craig, M., Fannin, N., Bush, L., Tobin, T., Electrospray tandem quadrupole mass spectrometry in the elucidation of ergot alkaloids chromatographed by HPLC: Screening of grass and forage samples for novel toxic compounds, *J. Mass Spectrom.* 2005, *40*, 1484 – 1502.

[33] Scott, P. M., Analysis of ergot alkaloids – a review, *Mycotox. Res.* 2007, *23*, 113 – 121.

[34] Bundesinstitut für Risikobewertung, *Mutterkornalkaloide in Roggenmehl*, http://www.bfr.bund.de/cm/208/mutterkornalkaloide_in_roggenmehl.pdf, 2004.

[35] Funk, W., Damman, V., Donnevert, G., *Qualitätssicherung in der Analytischen Chemie*, Wiley-VCH-Verlag, Weinheim 1992.

[36] DIN, *DIN 32645: Chemical Analysis; Decision Limit; Detection Limit and Determination Limit; Estimation in case of Repeatability; Terms, Methods, Evaluation*, Beuth Verlag, Berlin 1994.

[37] Storm, I. D, Have Rasmussen, P., Strobel, B. W., Hansen, H. C. B., Ergot alkaloids in rye flour determined by solid-phase cation-exchange and high-pressure liquid chromatography with fluorescence detection, *Food Addit. Contam.* 2008, *25*, 338 – 346.

[38] European Commission, Regulation (EC) No. 824/2000 of 19 April 2000 establishing procedures for the taking over of cereals by intervention agencies and laying down methods of analysis for determining the quality of cereals. *Off. J. Eur. Union* 2000, *L100*, 31 – 50.

[39] European Commission Decision 2002/657/EC of 12 August 2002 implementing Council Directive 96/23/EC concerning the performance of analytical methods and the interpretation of results. *Off. J. Eur. Union* 2002, *L221*, 8 – 36.

[40] Kromidas, S. (Ed.), *Handbuch Validierung in der Analytik*, Wiley-VCH-Verlag, Weinheim 2000, p. 74.

[41] Horwitz, W., Albert, R., Nesheim, S., Reliability of mycotoxin assays – an update, *J. AOAC Int.* 1993, *76*, 461 – 491.