FOOD CHEMICAL CONTAMINANTS

Determination of Ergot Alkaloids: Purity and Stability Assessment of Standards and Optimization of Extraction Conditions for Cereal Samples

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Results obtained from a purity study on standards of the 6 major ergot alkaloids ergometrine, ergotamine, ergosine, ergocristine, ergocryptine, and ergocornine and their corresponding epimers are discussed. The 6 ergot alkaloids studied have been defined by the European Food Safety Authority as those that are the most common and physiologically active. The purity of the standards was investigated by means of liquid chromatography with diode array detection, electrospray ionization, and time-of-flight mass spectrometry (LC-DAD-ESI-TOF-MS). All of the standards assessed showed purity levels considerably above 98% apart from ergocristinine (94%), ergosine (96%), and ergosinine (95%). Also discussed is the optimization of extraction conditions presented in a recently published method for the quantitation of ergot alkaloids in food samples using solid-phase extraction with primary secondary amine (PSA) before LC/MS/MS. Based on the results obtained from these optimization studies, a mixture of acetonitrile with ammonium carbonate buffer was used as extraction solvent, as recoveries for all analyzed ergot alkaloids were significantly higher than those with the other solvents. Different sample–solvent ratios and extraction times showed just minor influences in extraction efficacy. Finally, the stability of the ergot alkaloids in both raw cereals and cereal-based processed food extracts was studied. According to these studies, extracts should be prepared and analyzed the same day or stored below ambient temperatures. Barley and rye extracts, which were stored at 4 and 15°C after PSA cleanup, proved to be stable overnight. However, storage over a period of 14 days at 4°C resulted in significant epimerization, which was most pronounced in rye and particularly for ergocornine, ergocryptine, and ergocristine.

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The fungus *Claviceps purpurea* affects cereal crops and grasses, causing reduced yield and quality of grains and hay. It infects many hosts, including rye, triticale, wheat, durum, barley, oats, and various grasses, but is most prevalent in rye and triticale. More than 200 species of *Claviceps* are known (1). It is an important producer of toxic alkaloid metabolites (ergot alkaloids) in the overwintering fruiting body known as a sclerotium (2). Consumption of the toxins via cereal products or by livestock feeding on infected cereals or grasses causes the disease ergotism.

Ergot in cereals is subject to European Union (EU) Directive 2002/32/EC which establishes a maximum level for ergot bodies of 1000 mg/kg in all feedingstuffs containing nonground cereals (3). The EU Scientific Panel on Contaminants in the Food Chain of the European Food Safety Authority (EFSA) recently investigated ergot (4, 5) and concluded that a representative standard mixture of frequently occurring natural ergot alkaloids mixtures should be established. In addition, validated analytical methods for the quantitation of ergot alkaloids are needed as a prerequisite for

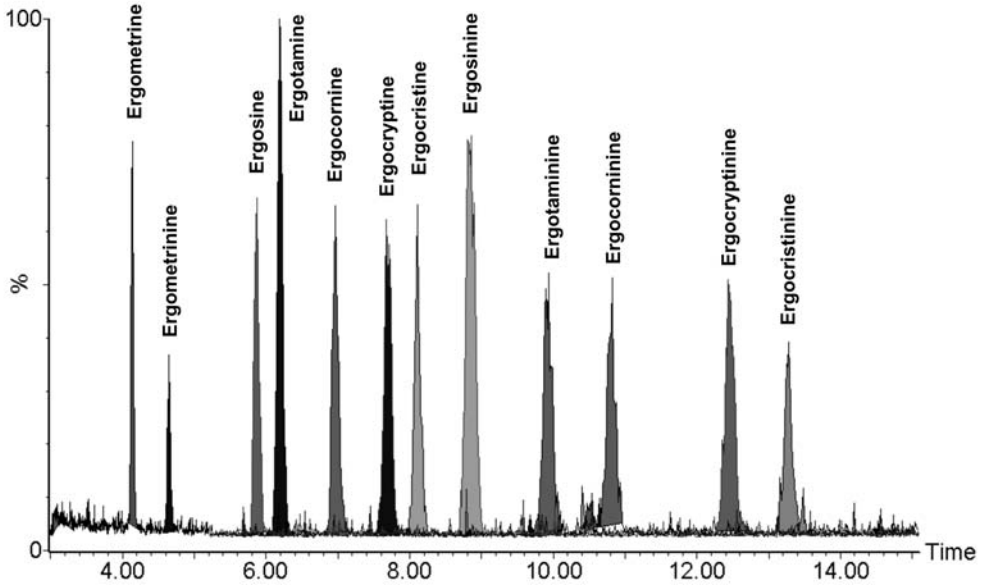

Figure 1. Overlaid LC/ESI(+)-MS/MS SRM chromatograms of 12 quantifier transitions (MH+=>223) at a spiking level of 1 μg/kg in wheat for each of the 12 ergot alkaloids (*see* ref. 22 for further details).

a survey on the occurrence of ergot alkaloids in food and feed materials in Europe. Analytical techniques should aim to detect the major ergot alkaloids as well as their corresponding biologically active metabolites formed in exposed animals.

There are 3 main groups of ergot alkaloids: the clavine type, the water-soluble lysergic acid type, and the water-insoluble lysergic acid type or peptide ergot alkaloids. The clavine type of alkaloids, such as agroclavine and elymoclavine, are generally regarded as precursors to the other groups of ergot alkaloids in the biogenetic pathway. By 1973, 46 ergot alkaloids from *Claviceps* spp. had been described (6), and since then several others have been characterized (7, 8). Ergot alkaloids containing a C9=C10 double bond readily exhibit epimerization, with the formation of a series of right-hand rotation (S)-isomers (9). The left-hand rotation isomers of ergot alkaloids [C-8-(R) configuration] are termed ergopeptines (e.g., ergotamine) and the right-hand rotation diastereomers are termed ergopeptinines (e.g., ergotaminine). Ergopeptinines always accompany ergopeptines in nature and can also be formed on

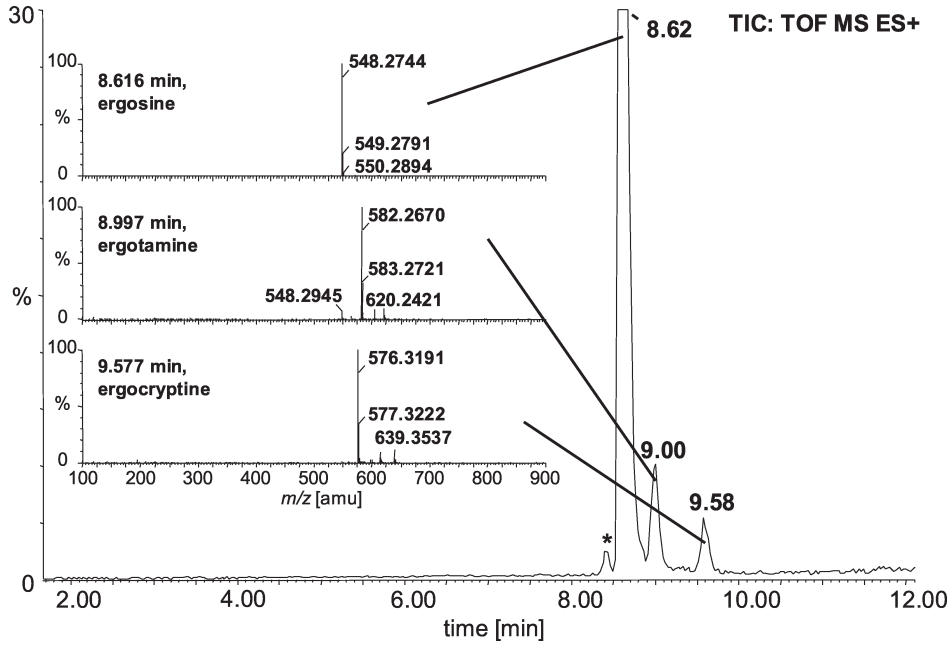

Figure 2. Total ion (ES+) chromatogram of the ergosine standard showing measured masses of the major peaks. The peak labeled with * is system-related.

Table 1. Characteristics of the ergot standards

Standard	Formula	Mass[a]	Ret. time, min	UV area	*m/z*	Error, ppm	Purity (UV, %)
Ergometrine	$C_{19}H_{23}N_3O_2$	325.1790	3.95	5.15E+05	326.1846	–6.9	99.8
Ergometrinine	$C_{19}H_{23}N_3O_2$	325.1790	4.91	6.85E+05	326.1866	–0.8	99.9
Ergosine	$C_{30}H_{37}N_5O_5$	547.2795	8.62	3.06E+05	548.2892	3.5	96.4
Ergosinine	$C_{30}H_{37}N_5O_5$	547.2795	8.26	8.82E+05	548.2866	–1.2	95.5
Ergocornine	$C_{31}H_{39}N_5O_5$	561.2951	9.06	2.60E+05	562.3011	–3.2	98.6
Ergocorninine	$C_{31}H_{39}N_5O_5$	561.2951	9.42	1.91E+06	562.3015	–2.5	99.5
Ergocryptine	$C_{32}H_{41}N_5O_5$	575.3108	9.58	4.18E+05	576.3172	–2.4	99.6
Ergocryptinine	$C_{32}H_{41}N_5O_5$	575.3108	9.90	1.02E+06	576.3197	1.9	99.4
Ergotamine	$C_{33}H_{35}N5O_5$	581.2638	8.98	4.78E+05	582.2722	1.0	99.1
Ergotaminine	$C_{33}H_{35}N_5O_5$	581.2638	8.74	1.43E+06	582.2711	–0.8	99.7
Ergocristine	$C_{35}H_{39}N_5O_5$	609.2951	9.90	1.95E+05	610.3025	–0.7	99.8
Ergocristinine	$C_{35}H_{39}N_5O_5$	609.2951	10.38	2.80E+06	610.3044	2.5	94.2

[a] Mono-isotopic mass (amu), neutral molecule.

storage of samples or during extraction from cereals. The C-8 epimers differ in biological and physicochemical properties, with the -ine forms being more active biologically (10, 11). Thus a small proportion of -inine alkaloids, based on isolysergic acid, are commonly reported in extracts of sclerotia. These may be generated, at least in part, during the extraction procedure through epimerization of lysergic acid (9).

The major ergot alkaloids ergometrine, ergotamine, ergocornine, ergocryptine, ergocristine, ergosine and their respective -inine isomers are frequently analyzed together by liquid chromatography (LC); the sum of the determined ergot alkaloids then is often referred to as the total alkaloid content (12). LC with mass spectrometric (MS) detection is now the most promising detection technique for ergot alkaloids in different matrixes and especially in feed and foodstuffs. The great advantages of this technique are that all known ergot alkaloids can potentially be determined in one run, provided that suitable standards are available, and that shorter chromatographic run time is needed, as co-elution is not a problem for compounds not showing overlapping MS/MS transitions. Furthermore, analogs not available as reference standards can be tentatively identified. The detection of a number of ergot alkaloids in grass by LC with tandem MS (LC/MS/MS) has only recently been described (13). Although only a few reports exist which describe the application of LC/MS/MS for detection of ergot alkaloids after their extraction into a liquid phase, the technique has many advantages. Compared to the more routinely applied fluorescence-based detection it is amenable to all forms (fluorescence detection cannot be used for analytes saturated at the C-10 position of the lysergic ring system, such as the clavine alkaloids). Furthermore, it may be used for structure confirmation and elucidation, which is of great importance because only a small number of all ergot alkaloids are well characterized, and the contribution of unknown compounds to the health hazard posed by food and feed contaminated by ergot alkaloids may be substantial.

There are challenging problems in the quantitative determination of ergot alkaloids in biological and environmental matrixes because of the reactivity of the analytes and the lack of suitable standards. A major problem is that solvent-, pH-, or temperature-induced epimerization occurs at the C-8 position of the lysergic ring system (13, 14), and the 2 resulting epimers, while chromatographically resolved on LC, show different relative intensities of the 2 common ergot alkaloid fragments ions *m/z* 223.2 and 208.2. This may be used as a diagnostic tool for the differentiation between the α- and the β-C-8 epimer (14) but it introduces an error, and therefore both peaks must be quantitated if LC/MS/MS in selected reaction monitoring (SRM) mode is applied (13). In addition, dehydration and hydrogenation of the analytes is suspected to occur during sample workup. Sample preparation methods include solvent extraction under alkaline conditions (to minimize epimerization) using chloroform (13, 15, 16), ethyl acetate (17), or methanol–water mixtures (14), followed by solid-phase extraction (SPE) or preparative LC for purification of the crude extract.

Reviews of available analytical methods for the determination of ergot alkaloids including the most frequently used LC/MS methods were published by Scott (18, 19), Komarova and Tolkachev (20), and most recently by Krska and Crews (21). Recently, a method developed and validated (22) for 10 different cereal and food samples enabled the quantitation of the 6 major ergot alkaloids defined by EFSA (ergometrine, ergotamine, ergosine, ergocristine, ergocryptine, and ergocornine) and their corresponding epimers (-inines). A fast cleanup based on dispersive SPE followed by a short chromatographic run (total run time

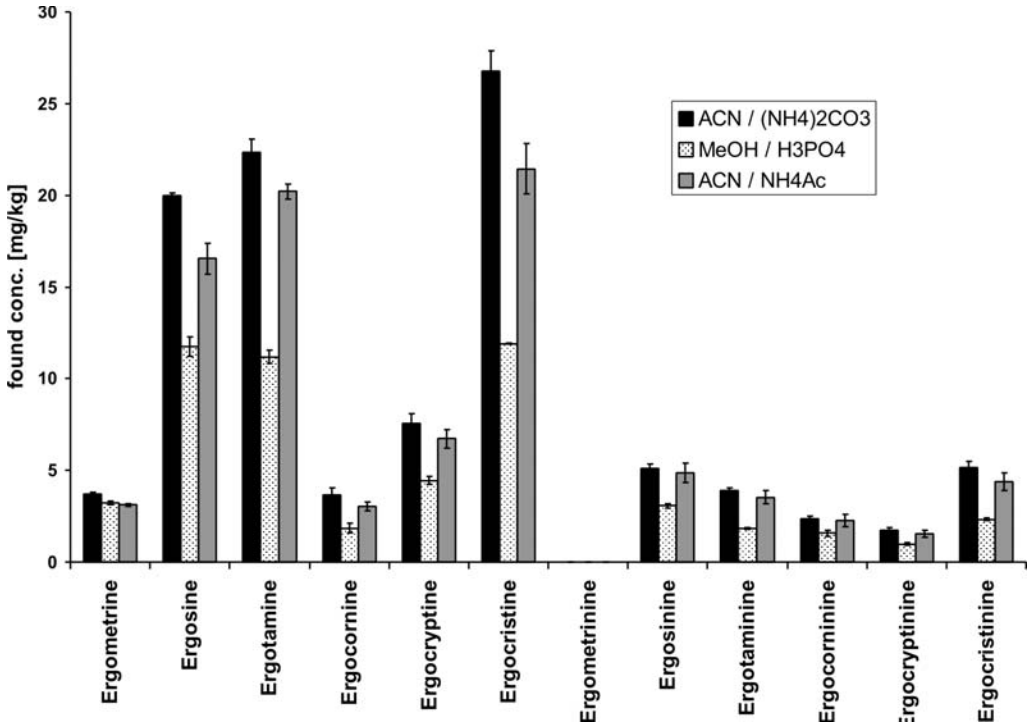

Figure 3. Extraction of ergots from barley (high level, *n* = 3) with alkaline, acidic, or neutral solvent mixtures.

21 min; Figure 1) and SRM in positive electrospray ionization [ESI(+)] mode resulted in limit of quantitation (LOQ) values of 0.17–2.78 μg/kg. Sulyok et al. (23) integrated 25 ergot alkaloids, including 5 ergopeptides and their epimers, in a multianalyte LC/MS/MS method without any prior cleanup, which in total covered 87 different mycotoxins. Though low limit of detection (LOD) values of 0.02–1.2 μg/kg have been achieved, a high degree of epimerization was observed in that study which strongly influenced the recoveries obtained for the individual ergot alkaloids.

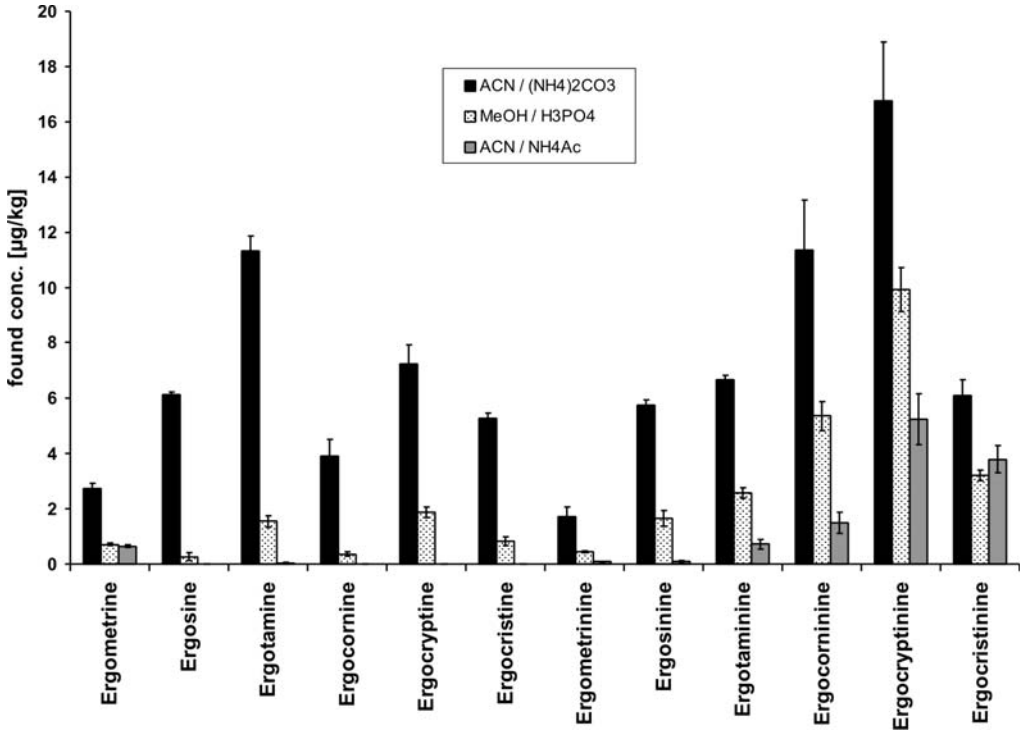

Figure 4. Concentrations of ergot alkaloids extracted from naturally contaminated rye (low level, *n* = 3) with alkaline, acidic, or neutral solvents.

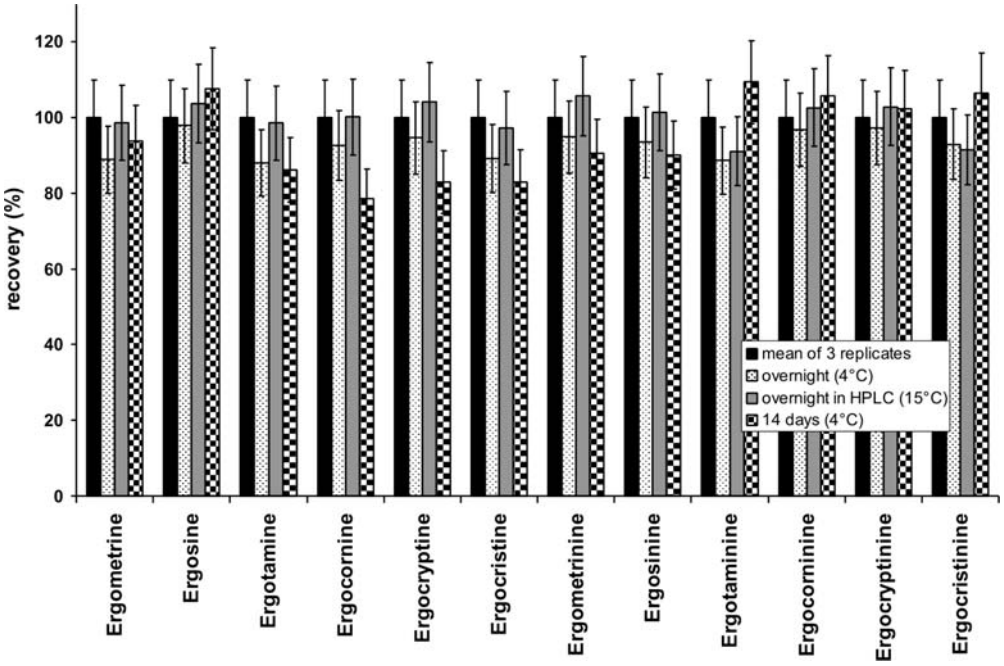

Figure 5. Stability of ergot alkaloids in barley extract after PSA cleanup.

The aim of this study was threefold: A major goal was the characterization of standards of 6 major ergot alkaloids and their corresponding epimers with respect to their purity; second, we observed the performance of different extraction conditions; and finally, we examined the stability of the dissolved ergot alkaloid in cereal extracts.

Experimental

Reagents

All chemicals were analytical grade and all solvents were HPLC grade. Crystalline standards of the ergot alkaloids ergocornine, ergocristine, ergocryptine, ergometrine (as hydrogenmaleate), ergosine, ergotamine, and its -inine forms

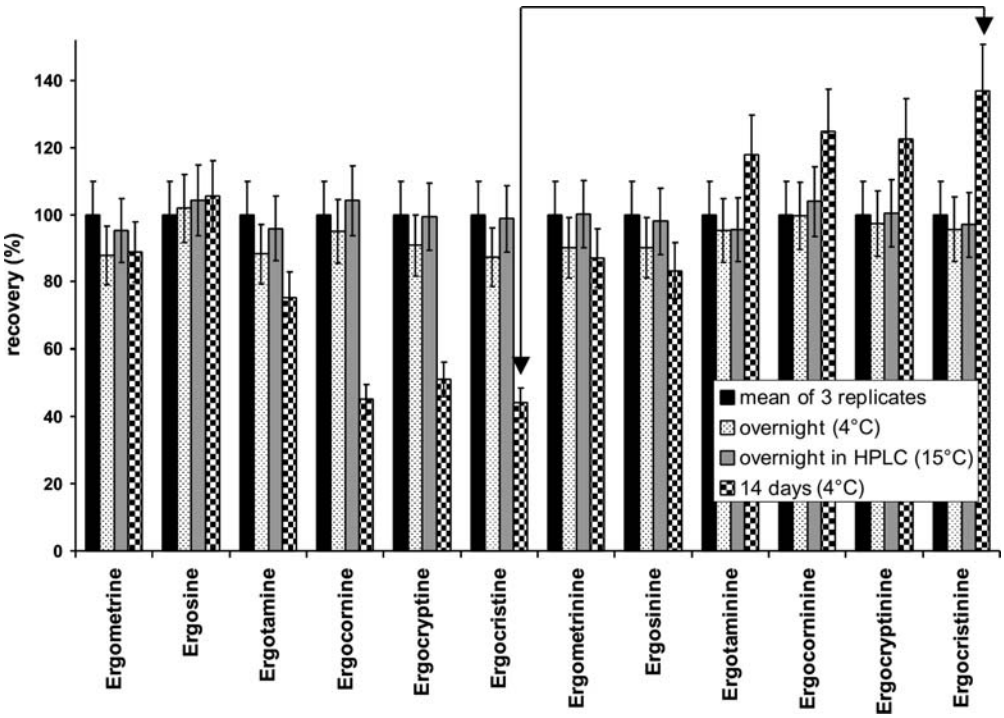

Figure 6. Stability of ergot alkaloids in rye extract after PSA cleanup. The arrow shows the partial conversion of ergocristine to ergocristinine.

were obtained from M. Flieger (Alfarma, Prague, Czech Republic). Cereal and cereal product samples were purchased from retail outlets.

Purity of the Crystalline Ergot Alkaloids

For purity determination, LC was performed on Agilent 1100 system (Waldbronn, Germany) using a Gemini C6-Phenyl (50 × 2 mm, 3 μm particle size) column (Phenomenex, Macclesfield, UK) at 40°C. An aqueous solution of 10 mM ammonium formate and 20 mM formic acid was used as eluent A, and acetonitrile containing 20 mM formic acid was used as eluent B. The flow rate was 0.3 mL/min. The linear gradient started at 5% B and reached 70% B after 18 min. Afterwards, the column was washed with 100% B for 25 min and re-equilibrated with 5% B until the end of the run at 36 min. The ergot solutions, in 3 μL acetonitrile, were injected into a flow of 300 mL/min. Diode array spectra were acquired from 200 to 700 nm with a range interval of 2 nm.

Electrospray positive spectra were acquired over the *m/z* range 100–900 using an LCT time of flight MS (Waters Ltd, Elstree, UK) connected in series with the diode array detector. The MS parameters were as follows: capillary 3000 V; sample cone 15 V; RF lens 200 V; extraction cone 5 V; desolvation temperature 450°C; source temperature 120°C; acceleration 200 V; microchannel plate (MCP) detector 2800 V; pusher frequency 20 000; ion energy 42 V; tube lens 3 V; TOF tube 4600 V; reflectron 1780 V.

Retention times and high-resolution molecular mass measurements were used as identification points for impurities and the standards. Antibase 2005, a microbial compounds database from John Wiley & Sons, Inc. (New York, NY) with about 30 000 entries, was searched for other *Claviceps* metabolites in case unknown compounds were present in the standards. A search window of 0.02 amu was used. Peaks visible in the UV chromatogram over the whole wavelength range were integrated to estimate the concentration of the impurities, assuming similar UV absorbance.

About 1 mg each of ergocornine, ergocristine, ergocryptine, ergometrine (as hydrogenmaleate), ergosine, and ergotamine, and their isomeric forms (ergocorninine, ergocristinine, ergocryptinine, ergometrinine, ergosinine, and ergotaminine) were carefully weighed into 8 mL glass vials using a microbalance. A 1 mL volume of pure acetonitrile was added to the standards and the vials were vortexed vigorously for 1 min. Then 30 μL of each solution was transferred into LC microvials already containing 270 μL acetonitrile to produce solutions of approximately 100 μg/mL. This concentration was chosen so that impurities down to 0.1% would still be detectable.

Optimization of Extraction Procedures

A mixture of acetonitrile and aqueous solvent (84 + 16, v/v) was used for the extraction of ergot alkaloids as recently described (22). Ammonium carbonate (200 mg/L, 3.03 mmol; pH 8.9 ± 0.3) was used as the aqueous phase of the extraction solvent mixture. The ground sample (5 g) was placed in a 60 mL amber sample jar with a PTFE screw-cap with 25 mL of the extraction solvent mixture and extracted for 30 min on a horizontal shaker. The extraction efficiency of acetonitrile–ammonium carbonate buffer (84 + 16, v/v) was compared with that of acidic methanol–0.25% phosphoric acid (40 + 60, v/v; 24), and with that of neutral acetonitrile–ammonium acetate solution (1 + 2, v/v; 23). A sample–solvent ratio of 5 g + 25 mL and an extraction time of 30 min were used throughout, with 3 replicate measurements made of each sample.

Barley naturally contaminated at a high level (up to 25 mg/kg ergot alkaloids) and a low level contaminated rye product (rye crispbread, up to 16 μg/kg) were used as commodities to test extraction efficiency. The extracts obtained were only diluted with the respective extraction solvent (rye 1:1, barley 1:50) and filtered before analysis, i.e., there was no cleanup step prior to the end determination by LC/MS/MS. Extraction with acetonitrile–ammonium acetate (1 + 2) required subsequent centrifugation at 15 000 rpm at 4°C for 30 min to enable separation of the sample from the solvent.

Quantitation of the 12 ergot alkaloids to determine extraction efficiency was performed using the validated LC-ESI(+)-MS/MS method of Krska et al. (22). In brief, separation was performed using a Gemini RP-C18 column 2 × 150 mm, 5 μm particle size (Phenomenex) fitted to an Alliance 2695 HPLC system (Waters) operated with an aqueous ammonium carbonate–acetonitrile gradient. The mass spectrometer was a Quattro Ultima Platinum tandem quadrupole instrument (Waters). MS/MS detection was performed in ESI(+) using the SRM acquisition mode.

Determination of Epimerization and Stability

The stability of the 6 ergot alkaloids and their 6 epimers in cereal extracts was tested on storage overnight at 4°C (refrigerator) at 15°C (LC autosampler, dark conditions) and over 14 days at 4°C. For that purpose, extracts of barley and rye were obtained by shaking 25 g sample with 100 mL solvent acetonitrile–ammonium carbonate buffer (200 mg/L; 84 + 16). The extracts were cleaned up using primary secondary amine (PSA), and then spiked at levels of 50 and 100 μg/kg, respectively. They were subsequently stored at the allocated temperatures until analysis by LC-ESI(+)-MS/MS (22).

Results

Purity Measurements

Predominantly $[M+H]^+$ ions were formed from the ergots and thus high-resolution MS was useful in proving the identity of the compounds. Masses measured matched the theoretical masses of the ergots extremely well (within 7 ppm). Detailed results for the standards (shown below in bold) were as follows:

Ergocristine (impurity at 10.56 min, 610.3082 amu, 485 mAU·min = 0.2% ergocristinine), **ergocryptine** (impurity at 10.06 min, 576.3200 amu, 1540 mAU·min = 0.4% ergocryptinine), and **ergometrine** (impurity at 4.53 min,

326.1876 amu, 1080 mAU·min = 0.2% ergometrinine) were very pure standards (>99%) and just showed minor impurities corresponding to the mass and retention time of their respective isomers in the chromatograms. While it cannot be ruled out that the isomers were formed in solution rather than being impurities in the solid standards, this seems unlikely, as in the other 3 measured ergots (ergocornine, ergosine, and ergotamine) no isomers were found.

Ergocornine contained both 0.8% ergocryptine (impurity at 9.64 min, 576.3128 amu, 2230 mAU·min) as well as 0.6% ergocryptinine (impurity at 9.88 min, 576.3183 amu, 1440 mAU·min), resulting in a total purity estimate of about 99%.

Ergosine contained one of the highest levels of impurities, with 2.7% ergotamine (impurity at 9.00 min, 582.2724 amu, 8480 mAU·min) and 0.9% ergocryptine (impurity at 9.58 min, 576.3191 amu, 3020 mAU·min), resulting in just 96% purity. A chromatogram of the ergosine standard, also showing the mass spectra of the major peaks is shown in Figure 2. The peak at 8.44 min, labeled with an asterisk *, is system-related and appears also in a blank. Mass spectra of the peaks are shown at the time of the highest intensity.

Ergotamine contained 2 more polar impurities at 7.90 min (598.2698 amu, 1140 mAU·min = 0.2%) and at 8.80 min (598.2642 amu, 3070 mAU·min = 0.6%). A search of the Antibase 2005 database resulted in both 8α-hydroxyergotamine and 8β-hydroxyergotamine as very likely hits. The total purity was estimated at 99%.

Ergocorninine was contaminated with 0.5% ergocryptinine (impurity at 10.08 min, 576.3191 amu, 8860 mAU·min) and with 0.1% ergocryptine (impurity at 9.94 min, 576.3242 amu, 1100 mAU·min). It therefore had a purity of >99%.

Ergocristinine was the most contaminated of all of the ergots, analyzed with an estimated purity of just 94%. The impurities comprised 1.7% ergocristine (impurity at 9.76 min, 610.3031 amu, 50900 mAU·min), 0.8% ergocorninine (impurity at 9.46 min, 562.3026 amu, 24100 mAU·min), and 3.3% ergocryptinine (impurity at 9.94 min, 576.3161 amu, 97000 mAU·min).

Ergocryptinine contained 0.1% ergocryptine (impurity at 9.42 min, 576.3191 amu, 1410 mAU·min) and a less polar compound 14 amu heavier than ergocryptinine, which is characteristic for methylation (impurity at 10.66 min, 590.3300 amu, 4520 mAU·min = 0.4%). According to Antibase 2005, the most likely identities are *O*-12′-methyl-α-ergocryptine, ergogaline, or ergoheptine. The purity of the ergocryptinine standard was therefore estimated as 99%.

Ergometrinine was extremely pure (99.9%), the sole less polar impurity being at 5.74 min (340.1993 amu, 419 mAU·min = 0.1%). This was not described in Antibase 2005 and thus was potentially a novel ergot alkaloid. The mass shift of 14 amu hints at a methylation of ergometrine or ergometrinine. The substitution of the sole methyl group with an ethyl group would also yield the same molecular mass, and this substitution is known from other ergots. MS/MS experiments could be performed to elucidate the structure of this minor impurity, but the very small amount of the substance in the standard might render these tests difficult.

Ergosinine contained both 2.0% ergosine (impurity at 8.50 min, 548.2866 amu, 18700 mAU·min) and 2.5% ergotaminine (impurity at 8.74 min, 582.2731 amu, 23100 mAU·min), giving a purity of 95% for the standard.

Ergotaminine (>99% purity) showed a single minor impurity at 9.42 min (596.2913 amu, 4030 mAU·min = 0.3%). According to the Antibase 2005 database the impurity could be ergostine, ergostinine, or the MW595 ethyl ergoxin group substituent described by Lehner et al. (13).

An overview of the results obtained from the characterization of the ergot standards is given in Table 1. This shows the formulas, monoisotopic masses (amu) for the neutral compounds, retention times (min), UV areas (mAU·min), measured *m*/*z* values of the protonated compounds (amu), calculated mass errors (ppm), and total purity (assuming similar UV absorbance of impurities, including isomeric forms). All of the standards investigated showed purity levels considerably above 98% apart from ergocristinine (94%), ergosine (96%), and ergosinine (96%). The purity of the ergot alkaloid standards was considered satisfactory, particularly in view of the limited sources and numbers of ergot alkaloid standards available and the purity of other commercial mycotoxin standards, which are usually between 95 and 99% (25). However, it has to be emphasized that certain impurities (like salts or inorganic materials) that do not have a chromophore, or which do not produce a signal in the range of the MS method are not detected by the method.

Extraction Procedures

Figures 3 and 4 demonstrate that the highest concentrations of the 12 ergot alkaloids tested were found after extraction with acetonitrile–ammonium carbonate buffer (84 + 16, v/v) for both matrixes, and particularly for the rye sample. To prove that the higher concentrations were the result of higher extraction efficiency rather than the result of matrix effects in MS (e.g., a signal enhancement effect), standard additions at a contamination level of 10 mg/kg ergot alkaloid for the highly contaminated barley were performed as described in more detail by Sulyok et al. (23). Reasonable recoveries for all 12 ergot alkaloids of 91–121% were obtained for these spiked samples and confirmed the high efficiency of the selected extraction mixture, while no significant matrix effect was determined.

Comparison of 3 different sample:solvent ratios (5 g sample to 15, 25, and 50 mL solvent) and 3 different extraction times (30, 60, and 90 min) were only performed for the most efficient extraction mixture acetonitrile–ammonium carbonate buffer (84 + 16, v/v). When investigating different sample:solvent ratios, different dilutions of 1:50 for 5 g barely + 25 mL solvent mixture, 1:25 for 5 g + 50 mL, and 1:82.5 for 5 g + 15 mL ensured comparable conditions for all 3 ratios with uniform end dilutions. After dilution the extracts were filtered, but no cleanup was performed before end determination by LC/MS/MS. Neither the investigated

sample: solvent ratio nor the extraction times significantly affected the extraction of the 12 ergot alkaloids from the rye samples. A very similar behavior was observed for the barley samples. However, for some ergot alkaloids a slight trend towards higher extraction efficiency with higher solvent sample ratio was observed. Based on the results obtained from the optimization of the extraction procedure, the following extraction conditions were chosen for all further analyses: 5 g sample + 25 mL acetonitrile–ammonium carbonate buffer (200 mg/L), 84 + 16 (v/v), 30 min extraction time.

Epimerization and Stability

Previous studies (26, 27) determined the epimerization of ergot alkaloids in organic and aqueous solvents and mixtures, indicating that aprotic solvents were more favorable for long-term stability of the toxins tested.

As can be seen in Figure 5, the barley extracts could easily be kept overnight at 4 or 15°C (in the dark) without any significant epimerization. However, when kept over a period of 2 weeks, epimerization of about 10% could be observed for ergocornine, ergocryptine, and ergocristine, even at 4°C.

A more pronounced but similar situation was observed for the rye extracts. As can be seen in Figure 6, the rye extracts could also be kept overnight at 4 or 15°C (in the dark) without any significant epimerization. However, when kept over a period of 2 weeks at 4°C, severe epimerization of ergocornine, >50%, was observed, but a high degree of epimerization also occurred for ergotamine, ergocryptine, and ergocristine. The decrease of -ines led to a corresponding increase in the corresponding -ininies as shown in Figure 6 for ergocristine/inine. According to these results, the degree of epimerization was obviously strongly dependent on the matrix. When extracts are stored for more than one night they should be maintained at temperatures below 4°C, at for instance –20°C, to minimize epimerization.

Conclusions

Standards of the 6 major ergot alkaloids ergometrine, ergotamine, ergosine, ergocristine, ergocryptine, and ergocornine and their corresponding epimers have been analyzed by LC-DAD-ESI-TOF-MS to determine their purity. While ergocristinine showed an estimated purity of 94%, ergosinine 95%, and ergosine 96%, all other standards had purity levels well above 98%.

Furthermore, extraction conditions able to maximize ergot alkaloid recovery, while minimizing epimerization, were elucidated. An optimum was found in extracting 5 g ground cereal sample for 30 min with 25 mL acetonitrile–ammonium carbonate buffer (200 mg/L), 84 + 16 (v/v). Alkaline extraction solvents were found to be necessary to obtain a high yield of ergot alkaloids from naturally contaminated rye. The extraction was followed by cleanup using PSA.

Finally, the stability of the ergot alkaloids in extracts of barley and rye was examined. We recommend preparation and analysis of the extracts on the same day or only very short-term storage at temperatures below ambient. Storage of extracts for 14 days at 4°C resulted in severe epimerization of some ergot alkaloids, especially ergocornine, ergocryptine, and ergocristine, and particularly in rye.

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