Journal of Chromatography A, 1441 (2016) 126–133

Contents lists available at ScienceDirect

Journal of Chromatography A

journal homepage: www.elsevier.com/locate/chroma

Screening for total ergot alkaloids in rye flour by planar solid phase extraction–fluorescence detection and mass spectrometry

Claudia Oellig*, Tina Melde

Institute of Food Chemistry, University of Hohenheim, Garbenstrasse 28, 70599 Stuttgart, Germany

ARTICLE INFO

Article history:
Received 1 January 2016
Received in revised form 22 February 2016
Accepted 24 February 2016
Available online 3 March 2016

Keywords:
Ergot alkaloids
Screening
Planar solid phase extraction (pSPE)
HPTLC
Fluorescence detection
TLC–MS

ABSTRACT

The analysis of ergot alkaloids is generally performed by high-performance liquid chromatography (HPLC) coupled to fluorescence detection (FLD) or mass selective detection. As for monitoring only the sum of ergot alkaloids is relevant, a fast and easy screening method for the determination of the total alkaloid content was developed using planar solid phase extraction (pSPE). Applying pSPE, recently introduced for pesticide residue analysis in fruits and vegetables (Oellig and Schwack, 2011) and tea (Oellig and Schwack, 2012), all ergot alkaloids are concentrated in a target zone followed by detection as the sum. The herein presented method includes an ammonium acetate buffered extraction step, followed by a fast liquid–liquid partitioning pre-cleaning before pSPE is performed on high-performance thin-layer chromatography (HPTLC) amino plates with a single methanol development to separate the ergot alkaloids from the remaining matrix and to collect them in a single zone. For quantitation, the native fluorescence was used after dipping the plate in *n*-hexane/paraffin solution for fluorescence enhancement. Limits of detection and quantitation of 0.07 and 0.24 mg/kg rye, respectively, expressed as ergocristine, were well below the currently applied quality criterion limit for rye. Near-100% recoveries were obtained at relevant spiking levels for different rye flour samples. Hence, the fast pSPE–FLD is an efficient and reliable method to screen for the total ergot alkaloid content in rye and a rapid alternative to the HPLC determination of individual alkaloids and to summing them up. HPTLC–MS additionally enables the identification of the ergot alkaloid composition by a single mass spectrum, when utilized as a fingerprint, offering an easy differentiation of *Secale cornutum* from different origins.

1. Introduction

Ergot alkaloids are produced by parasitic fungi from the *Claviceps* genus, commonly by the ergot fungus (*Claviceps purpurea*) [1], and are known to be responsible for different toxicological effects in mammals. Consumption of infested grain can lead to poisonings and cause strong disease [2,3]. Hence, the infestation of food and feed grain with ergots (*Secale cornutum*) as the permanent form (*sclerotia*) of *C. purpurea*, mainly growing on cereals, particularly on rye, is a serious problem. Chemically, ergot alkaloids are a group of about 50 indole alkaloids, commonly with an ergoline ring substituted at C-8 and methylated at N-6. The neutral ergot alkaloids are mainly derivatives of lysergic acid, with the six major alkaloids ergometrine, ergosine, ergocornine, ergocryptine, ergotamine, and ergocristine, also present as their isomeric inines [2,3].

Previously, a maximum level of 0.05% *S. cornutum* was laid down in regulation 2000/824/EC [4] for durum wheat, wheat and rye (bread grain or cereals). As according to regulations 2003/1784/EC [5] and 2005/1068/EC [6] the intervention on rye has been suspended, there exists a maximum value of 0.05% for *S. cornutum* in durum wheat and wheat according regulation 2009/1272/EC [7]. In regulation 2015/1940/EU [8] a maximum level of 0.05% *S. cornutum* is laid down for certain unprocessed cereals with the exception of corn and rice. For processed rye and rye flour, a maximum value of 0.05% *S. cornutum* furthermore applies as minimum quality criterion. Ergot alkaloids are counted as mycotoxins, thus, maximum levels should be laid down in regulation 2006/1881/EC [9]. However, individual ergot alkaloids are not regulated for grain and grain-based food, both on the European level and in Germany. Nevertheless, to limit the health risk, the European Union intended to minimize and regulate the total ergot alkaloid content of cereals and marketable food for a long time [10]. Monitoring ergot alkaloids in feed and food is strongly recommended by 2012/154/EU [11] and in regulation 2015/1940/EU [8], and a maximum level for a sum of the major ergot alkaloids in relevant food categories shall

* Corresponding author. Fax: +49 711 459 24096.
E-mail address: claudia.oellig@uni-hohenheim.de (C. Oellig).

http://dx.doi.org/10.1016/j.chroma.2016.02.075
0021-9673/

be considered soon [8]. In the United States, there is no maximum level established for ergot alkaloids in grain. In Canada, guideline levels for *S. cornutum* have been determined by the Canadian Grain Commission for the grading of cereal grains ranging from 0.01% for the highest quality grades, up to 0.1% for the lowest quality grades [12]. The basis to assess the marketability of rye and rye bread in Germany is the toxicological assessment of the Federal Institute for Risk Assessment (BfR) [13], which indicates 1000 μg/kg of total ergot alkaloids as "guidance level" for cereals, flour and cereal based foods. This assessment refers to article 14 of regulation 2002/178/EC [14] stating that food may not be placed on the market if it is harmful. The mentioned total is calculated regarding an ergot maximum level of 0.05% for durum wheat and wheat and the average content of 0.2% ergot alkaloids in pure *S. cornutum* in Central Europe, stated by the European Food Safety Authority (EFSA) [15]. In the actual opinion of EFSA (2012), a new calculation was performed for the average ergot alkaloid content in pure *S. cornutum* for Europe, resulting in 0.08% [12].

Ergot alkaloid analysis is performed by extraction with non-polar organic solvents under alkaline conditions [10,16–22] or with polar solvents under acidic conditions [23,24]. Different clean-up strategies utilize the basic properties of ergot alkaloids with acidic dissociation constants (pK_a) between 4.8 (ergocorninine) and 6.2 (ergometrinine) [3,25]. For clean-up, various methods exist including liquid–liquid partition [17] and solid phase extraction (SPE) or dispersive SPE (dSPE) with different materials and solvents. SPE is proposed by Mueller et al. working with alkaline alumina cartridges [18,19] or by Mohamed et al. applying octadecyl silica endcapped (C18 ec) [26], while Koeppen et al. [27] and Malysheva et al. [24] employed strong cation exchange (SCX) SPE cartridges. A fast clean-up strategy by dSPE with primary secondary amine (PSA) was shown by Krska et al. [20]. Determination is mostly performed by high-performance liquid chromatography (HPLC) coupled to fluorescence detectors (FLD) [10,19,21,27,28] or mass selective detectors (MSD), commonly tandem MS to gain high sensitivity [3,17,20,24,26,29].

Since for monitoring only the sum of ergot alkaloids is relevant and not the quantity of individual components, a meaningful new approach is the detection of alkaloids as the total. Therefore, the aim of the presented study was to develop a fast screening method for the determination of the total ergot alkaloids in rye by planar solid phase extraction (pSPE), which recently was developed by Oellig and Schwack as an efficient clean-up for pesticide residue analysis in fruits and vegetables and in tea samples, and offers the concentration of all target substances in a single zone, clearly separated from matrix components [30–32]. The adaption of pSPE to the rye matrix and to ergot alkaloids should be easily possible by variation of the sorbent and solvent system. For detection of ergot alkaloids as the total, simply the concentrated target zone needs to be measured, when the native fluorescence offers selective determination and high sensitivity. The use of fully-automated high-performance thin-layer chromatography (HPTLC) devices guarantees high efficiency and well repeatable procedures, when HPTLC–MS allows a rapid identification and determination of the alkaloid composition.

2. Material and methods

2.1. Chemicals and materials

Ergocristine (≥99%), ergotamine tartrate (≥98%) and ergometrine maleate (≥98%) was purchased from PhytoLab (Vestenbergsgreuth, Germany). Two *S. cornutum* samples, differing in alkaloid content, were provided by the State Plant Breeding Institute, University of Hohenheim (Stuttgart, Germany). Acetonitrile and methanol (both LC–MS, Chromasolv), toluene (for pesticide residue analysis), *n*-hexane (≥95%), gallic acid monohydrate (>98%, purum) and formic acid (for LC–MS, ~98%) were purchased from Sigma–Aldrich (Steinheim, Germany). Sodium chloride (≥99%, Ph. eur.), acetic acid (rotipuran, p.a.) and paraffin oil low viscosity (Ph. eur.) were obtained from Carl Roth (Karlsruhe, Germany). Ammonium acetate (≥99%, p.a.) was purchased from AppliChem (Darmstadt, Germany). Ultrapure water (>18 MΩ cm) was supplied by a Synergy System (Millipore, Schwalbach, Germany). HPTLC silica gel 60 NH_2 plates from Merck were prewashed with methanol and dried at room temperature inside a fume-hood for 30 min. The plates were stored in a SICCO Star-Vitrum desiccator (Bohlender, Grünsfeld, Germany) until use to prevent contamination.

2.2. Samples and extraction

As samples, different rye flours (German type 1150 and whole rye flour) and whole rye were purchased from a local supermarket. Whole rye flour and whole rye was finely milled in a Tube Mill control for 30 s at 12,000 min^{-1} (IKA, Staufen, Germany) before analysis. *S. cornutum* samples were also finely milled and sieved (<0.5 mm) before use. The ammonium acetate buffered extraction method according to Mohamed et al. [26] was used as a guideline for sample extraction with slight modifications. In brief, 6 g of ground sample was weighted into a 40-mL glass centrifuge tube, equipped with a screw cap and wrapped into aluminum foil. 10 mL acetonitrile and 20 mL 0.5 M ammonium acetate buffer pH 6.5 were added, and samples were extracted on a horizontal shaker for 30 min at 270 min^{-1} before being centrifuged at 3200 × *g* at 10 °C for 10 min (Biofuge primo R, Heraeus, Hanau, Germany). A 1-mL aliquot of the extract was transferred into a 6-mL glass centrifuge tube, equipped with a screw cap and wrapped into aluminum foil, and was horizontally shaken with 1 mL of toluene for 1 min at 2200 min^{-1} on a small-size shaking device (VXR basic, IKA, Staufen, Germany). For phase separation, 0.3 g sodium chloride was added and the mixture was shaken again for 1 min at 2200 min^{-1} before being centrifuged at 3200 × *g* at 15 °C for 5 min. The resulting gel mass in the toluene phase was slightly pushed to the wall of the centrifuge tube with a one-way laboratory spatula and a 300-μL aliquot of the clear toluene phase was transferred into an HPTLC vial with a 400 μL insert.

Spiked samples for recovery experiments were prepared by adding 1.5, 2 or 4 mg of finely milled *S. cornutum* to 6 g rye sample, corresponding to 0.5, 0.75, and 1.5 mg alkaloids/kg rye, calculated and expressed as ergocristine.

Amber glassware was used during the whole sample preparation or glassware was wrapped into aluminum foil.

2.3. Standard solutions

Standard stock solutions (40 mg/L) of ergocristine, ergotamine, and ergometrine were individually prepared in methanol. The stock solutions were stored at −20 °C in amber glassware and were useable for several weeks. For method development, stock solutions were diluted 1:10 with methanol or matrix extract, resulting in concentrations of 4 ng/μL for each alkaloid. To prepare the calibration standard solution, the ergocristine stock solution was diluted 1:150 with acetonitrile/0.5 M ammonium acetate buffer pH 6.5 (1:2, v/v), and the liquid–liquid partition with toluene was performed as described under Section 2.2. Thereafter, the volume of the upper toluene phase increased by 30%, thus leading to an additional dilution of 1:1.3 and resulting in a standard solution for application of 0.2 ng/μL.