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

C. Oellig, T. Melde and W. Schwack

Institute of Food Chemistry, University of Hohenheim

Scope

Ergot alkaloids are commonly produced by the ergot fungus *Claviceps purpurea* and are responsible for poisonings and toxicological effects in mammals. The parasitic fungus is mainly growing on cereals, particularly on rye, and the infestation of grain with *Secale cornutum*, the permanent form of *Claviceps purpurea*, is therefore a serious problem. About 50 ergot alkaloids from *Secale cornutum* are known, commonly derivatives of lysergic acid. The total alkaloid content varies considerably, depending on the origin between 0.01 and 0.5%, when a content of 0.2% is assumed for Central Europe. Despite the known toxicity and the infestation of rye grain and rye flours with *Secale cornutum*, there are no maximum limits established for ergot alkaloids in grain and grain-based food in Europe [1]. Nevertheless, the European Union strongly recommends the monitoring of ergot alkaloids in food and feed, and a maximum level for the total ergot alkaloid content of relevant food categories shall be considered soon. Since for monitoring the quantity of individual ergot alkaloids is not relevant, and only the sum of ergot alkaloids is monitored, the detection of ergot alkaloids as the sum is a meaningful and efficient new approach and offers the easy assessment of the exposure to ergot alkaloids. 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). pSPE was recently introduced as a clean-up method for pesticide residue analysis in fruits and vegetables and tea samples, offered the separation of target substances from matrix compounds and focused the target analytes in a single zone [2-4].

Procedure

Extraction of rye samples was done according to Oellig and Melde [5]. pSPE was performed on high-performance thin-layer chromatography (HPTLC) silica gel 60 NH_2 plates to fully separate ergot alkaloids and rye matrix components. After a single development with methanol, all alkaloids were focused into a sharp zone (same hR_F value), separated from the matrix (Fig. 1 A). Thereafter, ergot alkaloids were determined as the sum by a fluorescence scan at 254/>400nm after dipping into n-hexane/paraffin for enhancement (Fig. 1 B).

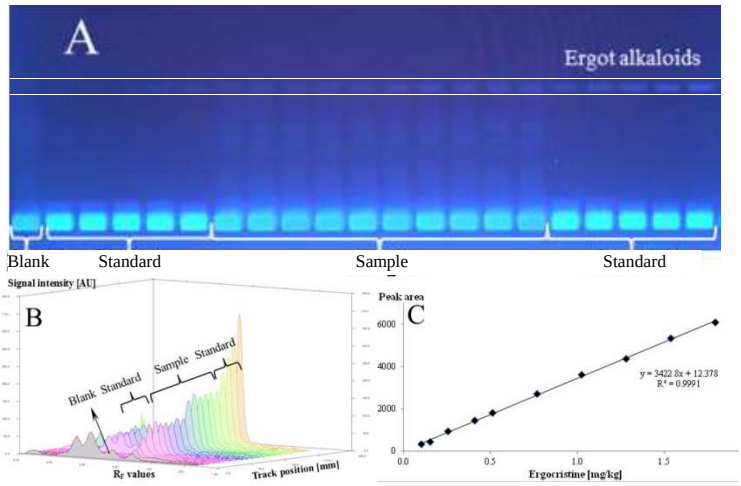

Figure 1 pSPE–FLD analysis: A) Plate image after pSPE on amino modified silica gel under UV 254 nm illumination of ergocristine, extracts of *Secale cornutum* spiked rye samples (German type 1150), spiking level 0.75 and 1.5 mg alkaloids/kg, expressed as ergocristine (n = 5), and a blank rye sample extract; (B) corresponding fluorescence scan at 254/>400 nm, and (C) resulting calibration graph of ergocristine, 0.4 – 7.2 ng/zone, displayed as 0.1 – 1.8 mg/kg rye [5].

Results and discussion

Method performance

Limits of detection (LOD) and quantitation (LOQ) were determined according to DIN 32645 calibration method [6], exemplarily shown in Fig. 1 C. LOD and LOQ were calculated to 70 and 240 µg/kg rye, respectively, expressed as ergocristine, which is well below the currently applied quality criterion limit for rye of 1000 µg alkaloids/kg rye. Recoveries for *Secale cornutum* spiked rye flours at spiking levels around the quality criterion limit were determined close to 100% with precision of recovery expressed as %RSD values below 4% (n = 5), which indicated the well repeatable pSPE–FLD analysis. Hence, the fast pSPE–FLD is an efficient and reliable method to screen for the total ergot alkaloid content in rye [5] omitting superfluous and time-consuming HPLC analysis of individual alkaloids.

Identification

For the identification of ergot alkaloids, HPTLC–MS of the target zone was easily performed by the TLC–MS interface (CAMAG). The zone was directly transferred into the MS and analyzed in less than one minute. A single mass spectrum extracted from the total ion chronogram contained the entire mass information of the ergot alkaloids present in a sample and their quantitative ratio, which enabled the rapid and easy identification of the alkaloid composition (Fig. 2).

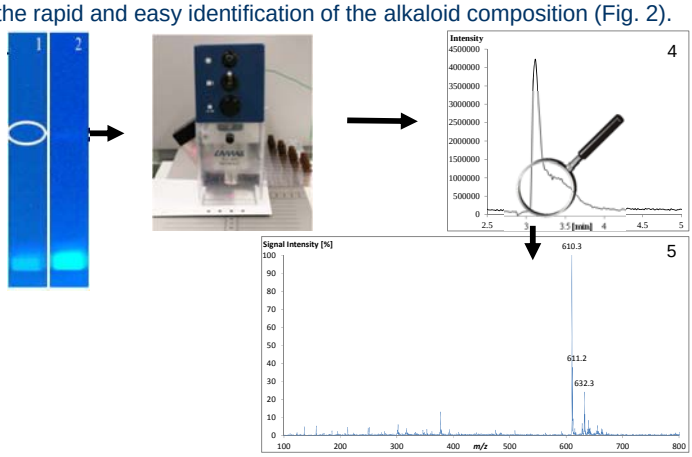

Figure 2 Identification of ergot alkaloids by HPTLC–MS, shown for ergocristine. (1) Ergot alkaloid zone after development with methanol under UV 366 nm illumination and (2) under UV 366 nm illumination after dipping in n-hexane/paraffin; (3) elution of the target zone by the TLC–MS interface (CAMAG); (4) total ion chronogram (ESI(+)MS) of the target zone and (5) extracted mass spectrum (*m/z* signals 610.3 [M+H]+ and 632.3 [M+Na]+ for ergocristine, 4 ng/zone, ~1 mg/kg rye).

Conclusion

- pSPE was again shown as an efficient method to separate co-extracted matrix components from target analytes and focus them into a sharp zone.
- pSPE–FLD is a fast and effective alternative to the time-consuming HPLC determination of individual alkaloids and calculating the sum.
- Method performance parameters with near-100% recoveries at relevant spiking levels were satisfactory and well repeatable, and sufficient sensitivity was additionally guaranteed.
- The identification of ergot alkaloids and their composition is easy possible by HPTLC–MS using the single mass spectrum as a "fingerprint".

Thanks to Merck (Darmstadt, Germany) for support with HPTLC plates and to the State Plant Breeding Institute, University of Hohenheim (Stuttgart, Germany) for providing ergot samples.

[1] BfR, Stellungnahme vom 7.11.2012 "Einzelfall-Bewertung von Ergotalkaloid-Gehalten in Roggenmehl und Roggenbroten"
[2] Oellig, C., Schwack, W., J. Chromatogr. A 1218 (**2011**) *6540-6547*
[3] Oellig, C., Schwack, W., J. Chromatogr. A 1260 (**2012**) *42-53*
[4] Oellig, C., Schwack, W., J. Chromatogr. A 1351 (**2014**) *1-11*
[5] Oellig, C., Melde, T., J. Chromatogr. A 1441 (**2016**) *126-133*
[6] Deutsches Institut für Normung, DIN 32645 Chemical analysis - Decision limit, detection limit and determination limit under repeatability conditions - Terms, methods, evaluation, Beuth, Berlin, 2008