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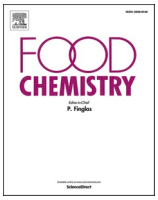

All-in-one 2LabsToGo system for analysis of ergot alkaloids in whole rye

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ABSTRACT

Ergot alkaloids, naturally occurring mycotoxins of *Claviceps* fungi, pose health risks. This necessitates accurate analysis methods to ensure food safety. This study explored the open-source miniaturized all-in-one 2LabsToGo system to analyze ergot alkaloids in whole rye samples. It is suited for sustainable atline analysis as it combines all planar chromatography tasks, allowing low-cost quality control in milling plants. The LOD and LOQ of ergocristine were determined to be 0.4 and 1.2 ng/zone, respectively. Detectability of ergot alkaloids was proven to be below the current maximum limit of 500 µg/kg for rye milling products. The repeatability (*%RSD*) was 4.1 % and the coefficient of determination of the analytical response (R^2) was 0.9918 for ergocristine. The mean recovery rate of ergot alkaloids in spiked whole rye grain was close to 100 %. Results of screening whole rye for ergot alkaloids were successfully verified by comparison with those obtained by conventional status quo HPTLC instrumentation.

1. Introduction

Ergot alkaloids are naturally occurring mycotoxins (Richard, 2007) produced by various species of parasitic fungi belonging to the genus *Claviceps*, especially by the ergot fungus *Claviceps purpurea* (EFSA Panel on Contaminants in the Food Chain, 2012). They have a long history of medical use and have been used as treatments for various conditions, including migraines (Friedman et al., 1959), Parkinson's (Sharma et al., 2016), and obstetrics (de Groot et al., 1998). However, ergot alkaloids also have toxic effects on humans and animals (Arcella et al., 2017) and can cause serious health problems leading to ergotism if consumed in higher amounts. Ergotism is a disease with symptoms including hallucinations, muscle pain, and gangrene which can lead to the loss of limbs and even be life-threatening. This has led to the regulation of ergot alkaloids in food and feed products and the need for accurate screening methods since analyzing the presence and levels of these alkaloids in cereals like rye is essential to ensure food safety and prevent health risks to consumers.

Rye and triticale are the cereals most infected by the *Claviceps* species, but wheat and other small grains can also be hosts of the parasitic fungus (Lorenz, 1979; Haarmann et al., 2009; Arcella et al., 2017). After infection, the grain or seed is replaced by the ergot sclerotia (*Secale cornutum*), the sclerotia containing the alkaloids, which is named ergot. The total ergot alkaloid content in the ergot sclerotia showed a high variation between 0.01 to 0.5 % (*w/w*) with different alkaloid patterns depending on the fungal strain (Krska et al., 2008). The infected and healthy cereals are harvested together, which is a source of ergot alkaloid contamination of food and feed products. The maximum limit for ergot sclerotia in unprocessed rye is 0.5 g/kg or 0.05 % (Commission of the European Communities, 2021). The maximum limit for ergot alkaloids in rye milling products available for the consumer was set to 500 µg/kg, expressed as the sum of twelve specified alkaloids (Commission of the European Communities, 2021). Exemplarily, for an assumed rye production yield of 5000 kg/ha, 2.5 kg ergot sclerotia/ha are allowed, which means 0.25–12.5 g/ha ergot alkaloids corresponding to 50–2500 µg/kg ergot alkaloids in rye. Although modern mill technologies can remove ergot sclerotia from the bulk grains, ergot dust adhered to the grains is nearly impossible to remove, so ergot alkaloids consequently contaminate milling fractions (Franzmann et al., 2011). Therefore, it is a good approach to check the cleaned grain for ergot alkaloids before the milling processes to guarantee the marketability of the produced flours.

Ergot alkaloids are chemically diverse derivatives of lysergic acid. The six major alkaloids produced by *Claviceps* species, *i.e.*, ergotamine, ergometrine, ergrocryptine, ergocristine (EC), ergocornine, and ergosine, all including their inine epimers (in total 12 alkaloids), are mostly targeted (EFSA Panel on Contaminants in the Food Chain, 2012; Krska &

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Crews, 2008; Flieger et al., 1997). Screening for ergot alkaloids in food and feed products is crucial in assessing their safety for human and animal consumption. Several analytical methods have been developed for detecting and quantifying ergot alkaloids, including high-performance liquid chromatography coupled to tandem mass spectrometry (Huybrechts et al., 2021; Mohamed et al., 2006) or fluorescence detection (Storm et al., 2008; Scott & Lawrence, 1980; Scott, 1993), and gas chromatography coupled to mass spectrometry (Clarkson et al., 1998). The complexity of grain samples requires extensive sample clean-up to remove interfering substances, especially when mass spectrometry is involved. Techniques such as liquid–liquid extraction (Di Diana Mavungu et al., 2012), solid-phase extraction (Köppen et al., 2013; Mohamed et al., 2006; Bryła et al., 2015), or dispersive solid-phase extraction (Krska et al., 2008) are commonly employed for clean-up. However, as the limits of ergot alkaloids are expressed as the sum of twelve species (Commission of the European Communities, 2021), it makes little sense to chromatographically separate and quantify all alkaloids and then sum them up. Hence, to directly quantify the sum of ergot alkaloids, a planar solid phase extraction method coupled to mass spectrometry was reported for milled rye products (Oellig & Melde, 2016).

In this study, before the milling processes, we propose to quantify all ergot alkaloids in the cleaned whole grain for utmost efficiency and low-cost analysis. The recently reported open-source all-in-one 2LabsToGo system was sought to be an ideal system for high-performance thin-layer chromatography (HPTLC) analysis (Sing et al., 2022). It is a sustainable, compact, portable, and miniaturized system, which was shown to provide comparable results to state-of-the-art HPTLC instrumentation (Schade et al., 2021). Recently, this was demonstrated for the screening and quantification of lactose-free dairy products and saccharide containing foods (Morlock et al., 2023). In this study, it was hypothesized that, compared to the status quo in HPTLC, a more cost-effective, sustainable, and simple atline screening of the ergot alkaloid limit in whole grain samples could be realized using the 2LabsToGo system. It could be used not only in analytical laboratories but also in milling plants to control the quality of exemplary rye before grinding. Thus, a 2LabsToGo method was developed and briefly validated, and the results were verified by comparison with those obtained from state-of-the-art HPTLC devices.

2. Materials and methods

2.1. Chemicals and materials

HPTLC plates silica 60 NH_2 (20 cm × 10 cm, cut to 10 cm × 10 cm format via TLC Plate Cutter, CAMAG, Muttenz, Switzerland) and paraffin oil were obtained from Merck (Darmstadt, Germany). Bidistilled water was obtained using a Destamat Bi 18E (Thermo Fisher Scientific, Dreieich, Germany). Ergocristine (EC) was purchased from PhytoLab (Vestenbergsgreuth, Germany). The finely milled ergot sclerotia sample was provided by the State Plan Breeding Institute, University of Hohenheim (Stuttgart, Germany). Acetic acid (99–100 %), acetonitrile (100 %), ethanol (>99.7 %), methanol (100 %), and *n*-hexane (≥97 %) were from VWR (Darmstadt, Germany). Toluene (≥99.9 %) and gallic acid monohydrate (≥98 %) were delivered by Carl Roth (Karlsruhe, Germany), and ammonium acetate by Sigma-Aldrich (Taufkirchen, Germany). Whole rye samples (R1: Alnatura, Darmstadt, R2: Demeter, Schwalmtal-Hopfgarten, R3: Antonius, Fulda, R4 and R5: Bohlsener Mühle, Bohlsen, all from Germany) were purchased from local supermarkets and stored in the original package at room temperature in the dark.

2.2. Sample extraction, spiking of samples, and EC standard solution

The extraction of ergot alkaloids from rye with ammonium acetate buffer and acetonitrile (Oellig & Melde, 2016; Mohamed et al., 2006) was slightly modified by adding gallic acid to the buffer since ergot alkaloids are prone to photo-oxidation. Briefly, each whole rye sample (10 g) was weighed into a 50-mL Falcon tube wrapped in aluminum foil for light protection. A mixture of ammonium acetate buffer (10 mL, 6.9 mM, pH 6.5, containing 0.1 % gallic acid) and acetonitrile (5 mL) were added to the samples as extraction buffer, which were shaken for 10 min at level 7 (Vortex-Genie 2 with multi-tube holder, Scientific Industries, NY, USA) and centrifuged for 10 min (3000 × g, Labofuge 400, Heraeus, Hanau, Germany). From each extract (10 g/15 mL), five aliquots of 2 mL were transferred into Eppendorf Safe-Lock-Tubes wrapped in aluminum foil and centrifuged again for 10 min (13,000 × g, Heraeus Pico 17 Microcentrifuge, Thermo Fisher Scientific, Langenselbold, Germany). The supernatants were collected in amber glass vials and analyzed directly (optionally stored at 4 °C up to three days).

For recovery experiments, three aliquots of the rye sample R1 (10 g each) were spiked each with 2.22 mg finely milled ergot sclerotia containing 2.25 g/kg EC equivalents (Oellig & Melde, 2016) to reach the 500-µg/kg maximum limit (5 µg EC per 10 g) and simulate ergot dust on the surface of whole rye grain, shaken and prepared as mentioned. The ergot sclerotia extract was prepared by dispensing 2.22 mg finely milled ergot sclerotia in 15 mL extraction buffer followed by ultrasonication for 15 min, centrifugation for 10 min (13,000 × g), and transfer of the supernatant in an amber glass vial.

Stock solution of EC (1 mg/mL) was stored at −20 °C in an amber glass vial. The EC standard solution (333 pg/µL) was obtained by 1:3000 dilution with the extraction buffer.

2.3. Method using open-source 2LabsToGo system

The functionality of the latest open-source 2LabsToGo hardware (free download at https://github.com/OfficeChromatography/OCLab3) and software (free download at https://github.com/OfficeChromatography/OC-Manager3) has been described elsewhere (Sing et al., 2022). Each HPTLC plate silica gel 60 NH_2 was prewashed with the mobile phase up to 60 mm using the same parameters as for the development and dried for 2 min with a cold stream of air (hair dryer). Solutions were applied as 5 mm × 4 mm areas (distance in between 3 mm, from the plate bottom 10 mm, and from both sides 9 mm). The EC standard solution (333 pg/µL) was applied ten times at 10.0 µL each for repeatability (3.3 ng/area corresponding to 500 µg/kg ergot alkaloids in rye, respecting the method of sample preparation) and in increasing volumes for the analytical response study (1.5, 2.5, 4.0, 5.0, 6.5, 7.5, 10.0, 12.5, 15.0, 17.5, 20.0, and 25.0 µL/area, resulting in 0.5, 0.8, 1.3, 1.7, 2.2, 2.5, 3.3, 4.2, 5.0, 5.8, 6.7, and 8.3 ng/area, corresponding to 75, 125, 200, 250, 325, 375, 500, 625, 750, 875, 1000, and 1500 µg/kg ergot alkaloids in rye, respecting the method of sample preparation). For both the recovery study and sample screening, the sample extracts, EC standard solution (333 pg/µL) and blank extracts were applied (all 10.0 µL each). The following parameters were set for application with the Atomizer 67 k nozzle: motor speed 2000 mm/min, pressure 25 psi, frequency 1400 Hz, delta X/Y 0.75, temperature 60 °C, and rinsing period 100. Before application, a valve warmup of 750 droplets (~25 µL) with 25 psi (G97 P25) and 1400 Hz (G98 F1400) was performed into the waste.

To ensure a dry environment during the development, a hot and scratched HPTLC silica gel 60 plate was used as cover plate instead of the normally used glass plate cover. Briefly, the adsorbent was scraped off with a spatula to create a transparent centerline of 2 mm × 100 mm through which the migration of the solvent front was observed. Thereafter, the plate was heated at 110 °C for 20 min (heatable plate holder) and placed hot with the layer faced downwards on top of the development chamber cover frame. For 10 min, the humidity inside the horizontal chamber was allowed to adsorb, increasing the activity of the chromatography plate underneath. Then, the latter plate was horizontally developed up to a migration distance of 40 mm by moving the nozzle back and forth in the x-direction (as an imaginary line) along the

plate overhang, delivering a continuous jet with 2 mL ethanol – toluene 4:1 (*V/V*) as mobile phase. The following parameters were set for mobile phase dosage with the Atomizer 67 k nozzle: plate offset left/right − 2 mm, top/bottom 1 mm, motor speed 35 mm/min, pressure 30 psi, 50 applications, estimated flow rate 20 µL/s, print both ways (yes), and dynamic waiting times (5 − 60 s, Table S1). After development, the plate was dried for 2 min with a cold stream of air (hair dryer).

For fluorescence enhancement of the ergot alkaloids, 1.7 mL *n*-hexane – paraffin oil (2:1, *V/V*) were applied onto the plate via the solution-dispenser trough (Instruction S1), whereafter the plate was left to dry for 2 min at ambient temperature. For detection, the plate image was recorded at 255 nm illumination by a light-emitting diode (turn on LED with G-Code M42P5S100) using raspistill (shutter speed (−ss) 2000000, brightness (−br) 50, ISO (−ISO) 800, white balance (−awb) auto). Raspistill is a Linux command to control the Raspberry Pi HQ camera, but because of missing python bindings it could not be included into the OCManager3 software. To simplify taking pictures with raspistill, a bash script was written (Instruction S2). For digital image evaluation, the open-source software quanTLC (Fichou & Morlock, 2018) was used (freely accessible at https://sv09010.ernaehrung.uni-giessen.de/qua

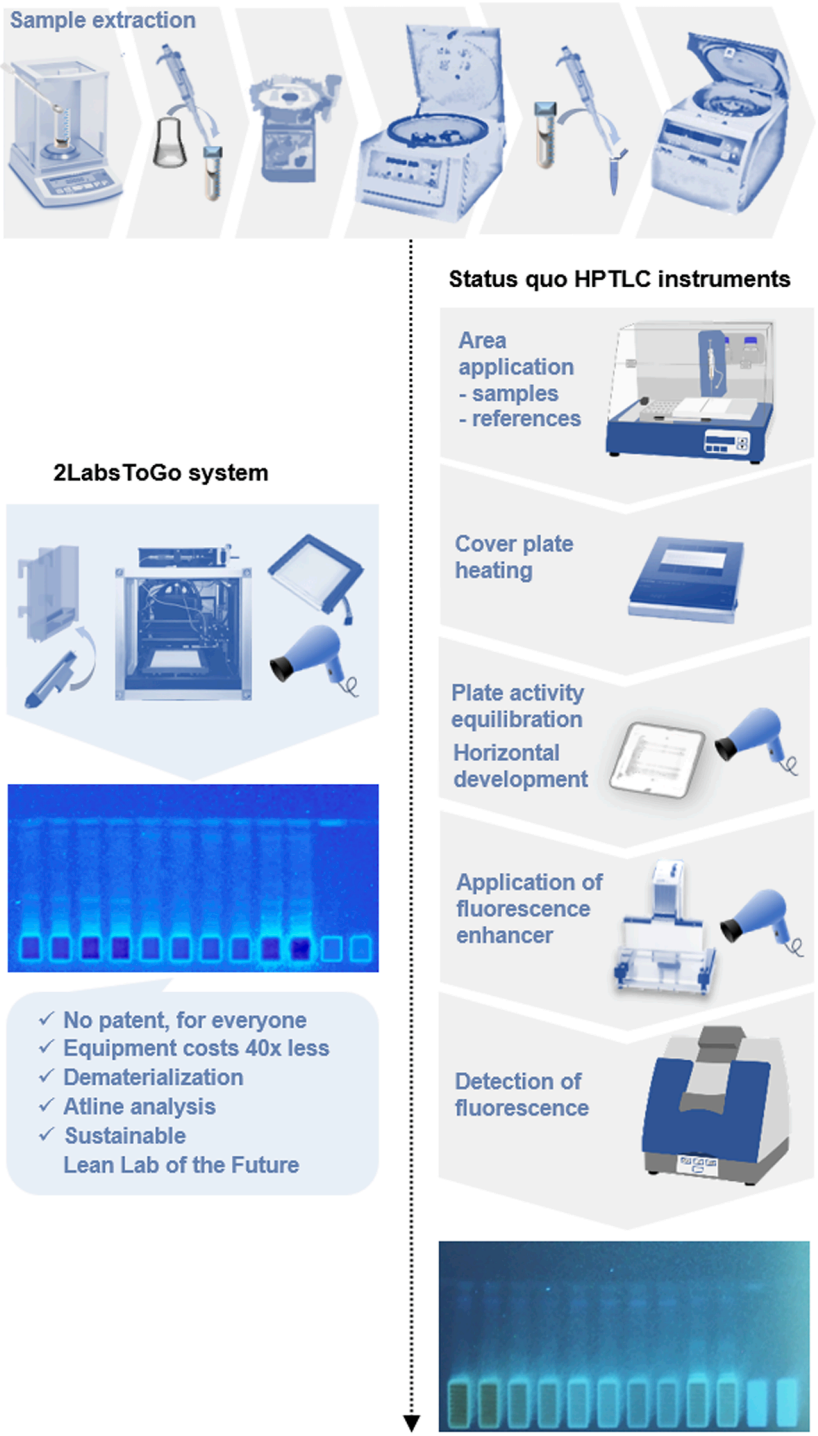

Fig. 1. Schematic comparison of two different instrumental technologies (adjusted from Morlock et al., 2023) for screening of whole rye products for ergot alkaloids as well as benefits of the 2LabsToGo system.

nTLC) selecting the green channel (Instruction S3). Briefly, for each sample track, the pixels of each line were automatedly summed up, generating a videodensitogram curve along the separated sample track. The resulting area under the peak (or peak height) can be treated and evaluated in a similar way to an ordinary densitogram.

2.4. Method using conventional status quo HPTLC instruments

The conventional instruments (CAMAG, Muttenz, Switzerland) were controlled with visionCATS software version 3.2.22308.1. Each HPTLC plate silica gel 60 NH_2 was prewashed up to 60 mm with the mobile phase and dried (TLC Plate Heater). The parameters were used in analogy to the 2LabsToGo method, *i.e.*, sample and blank extracts (10.0 µL each) and EC standard solution (1.5 − 25.0 µL) were applied as areas of 4 mm × 5 mm (Automatic TLC Sampler 4, ATS 4) onto the HPTLC plate and developed with 3 mL ethanol – toluene 4:1 (*V/V;* Horizontal Development Chamber, HDC). Larger areas of 4 × 9 mm were applied for the initial screening plate, where also matrix effects were studied (Fig. S1), but the application area was then reduced to be more compact because there was no difference between the area geometries of 4 mm x 5 mm and 4 mm x 9 mm. Analogously for a dry chamber climate during the development, an HPTLC silica gel 60 plate cut to 80 mm × 85 mm (TLC Plate Cutter) was heated at 110 °C for 20 min (TLC Plate Heater) and placed hot with the layer faced upwards on the bottom of the inner HDC trough. Immediately, the HPTLC plate with the samples was inserted with the layer facing downwards and the HDC glass cover on top. After humidity adsorption for 10 min, the development was started. Reaching a migration distance of 40 mm, the plate was taken out and dried for 2 min with a cold stream of air. For fluorescence enhancement, the chromatogram was immersed in *n*-hexane – paraffin oil 2:1 (*V/V*; TLC Immersion Device III, vertical speed 2.5 cm/s, immersion time 3 s) and allowed to dry for 2 min. Plate images were captured at 254 nm (TLC Visualizer 2), and for instrumental comparison, evaluated with the same quanTLC software (Instruction S3).

3. Results and discussion

3.1. Development of the 2LabsToGo method

Using the latest 2LabsToGo system (Sing et al., 2022; Morlock et al., 2023), a method was developed for a cost-effective, sustainable, and simple on site screening of whole rye samples for ergot alkaloids (Fig. 1). The EC belongs to the major alkaloids in ergots and was selected as a reference standard to represent the group of ergot alkaloids. Ergot alkaloids have a common Δ9 conjugated indole chromophore, lysergic acid, responsible for the fluorescent properties. The differences in structure result from different amino acids building the almost tricyclic tripeptides linked to lysergic acid by an amide bond, but they are not part of the π system. Therefore, the light absorption and emission characteristics are expected to be identical for the twelve regulated alkaloids on the molar basis. Bowd et al. (1973) studied the "luminescence characteristics of lysergic acid diethylamide and related ergolines". For ergometrine, ergotamine, EC (icluding their inines), and lysergic acid, they determined nearly the same molar absorptivities. Slight differences may be explained by the standard purities. The EC can therefore be used as a representative.

Five different rye samples were bought to represent the variability of rye matrix. Since ergot alkaloids are prone to photo-oxidation, gallic acid was added to the extraction buffer (Mohamed et al., 2006), analogously to its application on the start zone (Oellig & Melde, 2016). Thus, the extraction was performed with a 2:1 (*V/V*) mixture of sodium acetate buffer containing 0.1 % gallic acid and acetonitrile. The rye extracts were applied as 4 mm × 5 mm areas due to the high matrix load. Methanol was first tested as mobile phase, but rather broad zones were observed, presumably due to horizontal development as opposed to vertical development (Oellig & Melde, 2016). Therefore, methanol was substituted by ethanol reducing the migration speed, and finally with the addition of 20 % toluene all ergot alkaloids migrated as sharp zone near the solvent front, well separated from matrix compounds (Fig. 2). Additionally, the zone sharpness was increased by reducing the humidity in the development chamber. The glass plate normally inserted in the frame of the cover of the 2LabsToGo development chamber was replaced by a line-scratched and hot (thus dry) silica gel plate with the layer facing downwards. This increased the activity of the underlying HPTLC plate, which contained the applied samples, and resulted in the desired sharp zones after development compared to a glass plate cover or solvent-wet silica gel plate cover.

The native blue-green fluorescence of ergot alkaloids could be enhanced threefold by applying a mixture of *n*-hexane – paraffin oil 2:1 (*V/V*) onto the plate using the solution-dispenser trough of the 2LabsToGo system. Illumination at 255 nm in the 2LabsToGo system, which is close to the absorption maximum at 260 nm of ergot alkaloids (as determined for EC on the HPTLC plate) allowed the sensitive detection of the sum of ergot alkaloids in a single blue-green fluorescent zone near the solvent front by the Raspberry Pi HQ camera. The zone assignment was confirmed by applying an EC standard and an ergot sclerotia reference extract (Fig. 3). The ergot alkaloids were detectable even at the lowest applied amount of the EC standard (0.5 ng/area) corresponding to 75 µg/kg ergot alkaloids in rye, providing initial evidence of the method's sensitivity. The obtained 2LabsToGo plate images were digitally transformed into videodensitograms and evaluated using the open-source software quanTLC (Fichou & Morlock, 2018) (Instruction S3).

3.2. Brief method validation in comparison to the status quo HPTLC instruments

To evaluate the repeatability of the analysis, the EC standard solution was applied ten times (10 µL or 3.3 ng/area each, corresponding to 500 µg/kg ergot alkaloids in rye). The 2LabsToGo method was performed as described, while the conventional analysis used status quo HPTLC instrumentation consisting of autosampler for sample application (ATS 4), horizontal development chamber (HDC), TLC Plate Heater, TLC Immersion Device III, and TLC Visualizer 2. For both systems, image evaluation was carried out with the open-source quanTLC software. The repeatability of the EC analysis (n = 10, Fig. 2) with the 2LabsToGo system (*%RSD* of 4.1 %) was highly satisfying and comparable to the results obtained with the status quo HPTLC instruments (*%RSD* of 4.8 %).

The limits of detection (LOD) and quantification (LOQ) of the EC analysis via the 2LabsToGo system were determined via the calibration curve method (ICH, 1995/2005) to be 0.4 and 1.2 ng/zone, corresponding to 60 and 180 µg/kg ergot alkaloids in rye, respectively. It was comparable to the LOD and LOQ of 0.5 and 1.4 ng/zone, corresponding to 75 and 180 µg/kg ergot alkaloids in rye, respectively, obtained by the status quo HPTLC instruments. To study the analytical response, the EC standard solution was applied at twelve different volumes in the range of 1.5–25.0 µL (0.5–8.3 ng/area, corresponding to 75–1250 µg/kg ergot alkaloids in rye). The coefficient of determination of the analytical response obtained via linear regression by the 2LabsToGo system (R^2 of 0.9918) was comparable to that of the status quo HPTLC instruments (R^2 of 0.9890, Fig. 2).

The recovery rates of ergot alkaloids determined three times at the maximum limit of 500 µg/kg for processed rye products (Commission of the European Communities, 2021) were studied using the whole rye sample R1 (visibly free from any ergot sclerotia or sclerotia fragments) spiked with a powdered ergot sclerotia sample certified to contain 2.25 g/kg EC equivalents (Oellig & Melde, 2016). This should simulate ergot dust on the surface of whole rye grain. The mean recovery rates obtained were near 100 % for all three spiked samples (Fig. 3). Recovery rates slightly above 100 % can be explained by the possible presence of ergot alkaloid traces in the sample, however, far below the selected benchmark of 500 µg/kg. The good recovery rate and the well-reached

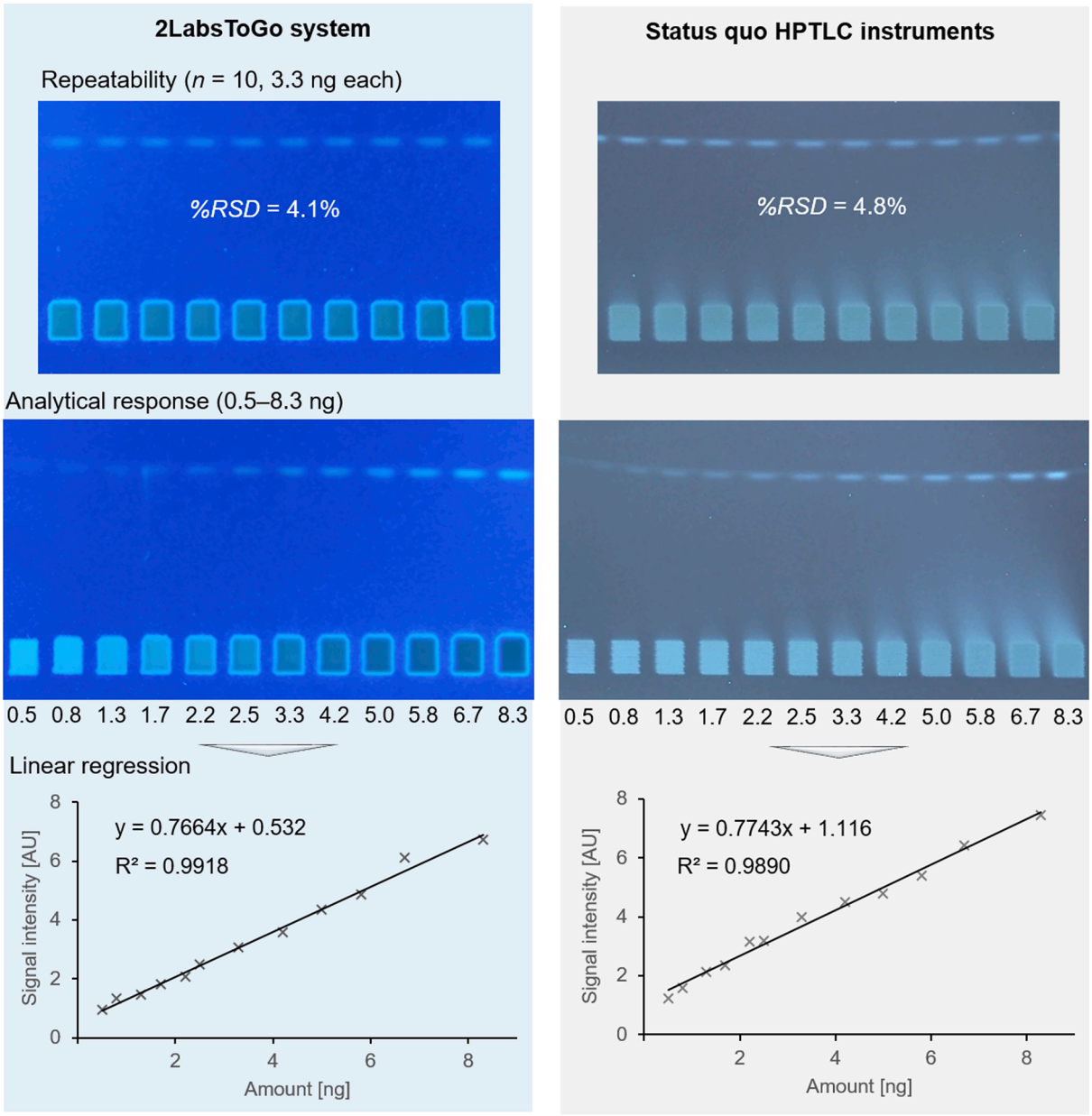

Fig. 2. Comparison of repeatability (10.0 μL each) and analytical response (1.5, 2.5, 4.0, 5.0, 6.5, 7.5, 10.0, 12.5, 15.0, 17.5, 20.0, and 25.0 μL) for EC via the 2LabsToGo system *versus* conventional status quo HPTLC instruments, applied as 4 mm × 5 mm area on the HPTLC plate silica gel 60 NH_2, developed with ethanol – toluene 4:1 (*V/V*) up to a migration distance of 40 mm, detected at 255 nm (2LabsToGo system) or 254 nm (status quo TLC Visualizer 2) after fluorescence enhancement with *n*-hexane – paraffin oil 2:1 (*V/V*) and evaluated using open-source quanTLC software; the strong fluorescence on the start area is caused by the gallic acid used as antioxidant in the extraction buffer; the blue background can be adjusted to black in the latest updated OCManager3 software (not used here). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

maximum limit proved successfully the simple surface extraction and planar chromatography, which was to be expected due to the minimalist sample preparation (simply extraction of the grain surface and centrifugation) without the slightest clean-up step. A matrix effect can be neglected using this method. The co-extracted matrix was visible in the start zone, clearly separated by the mobile phase from the ergot alkaloids.The latter were eluted and focused as single target zone close to the solvent front. The application in form of areas instead of bands also minimized matrix effects. All in all, the comparative validation data proved and verified the suitability and performance of the developed 2LabsToGo screening method for detecting the sum of ergot alkaloids in whole rye samples. The developed method can be regarded as the first step of using the 2LabsToGo system for the atline screening of ergot alkaloids in whole rye. Further in-house validation need to follow.

3.3. Screening of ergot alkaloids in whole rye products

Due to the toxicity of ergot alkaloids in contaminated rye, the need for screening is obvious. The sum of ergot alkaloids was determined after a simple surface extraction, neglecting the otherwise required chromatographic separation and summing up of individual results. Such an HPTLC approach has been developed for the analysis of milled flour products (Oellig & Melde, 2016). However, in contrast, the whole rye can be analyzed in milling plants before the milling processes, which is most efficient. In the Regulation (EU) 2021/1399 amending Regulation (EC) 1881/2006, a maximum limit of 150 μg/kg was set for ergot alkaloids in whole grains of barley, wheat, spelt, and oat placed on the market for the final consumer, whereas it was 500 μg/kg for ergot alkaloids in rye milling products (Commission of the European

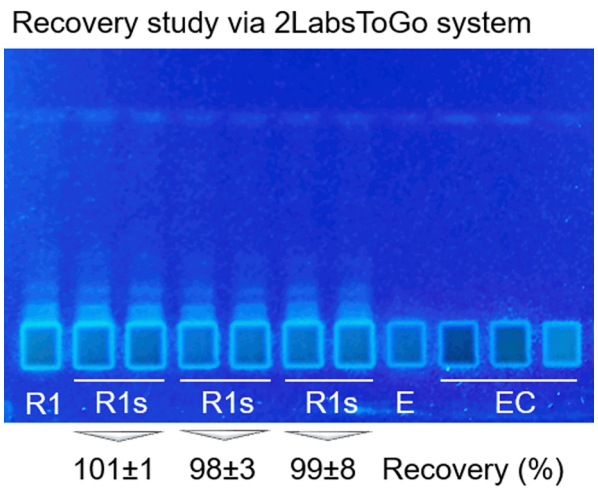

Fig. 3. Recovery rate of ergot alkaloids in whole rye analyzed via the 2LabsToGo system: three independent extracts of the same whole rye sample R1, each spiked with certified ergot sclerotia powder at 500 μg/kg (R1s), extracted and analyzed two-fold (10.0 μL each) as in Fig. 2, along with references ergot sclerotia extract (E, 10.0 μL) and EC standard solution (5.0, 10.0 and 15.0 μL, 1.7 – 5.0 ng/area) as well as extraction buffer blank (B, 10.0 μL).

Communities, 2021). The latter maximum limit was considered also for whole rye in the present study. Thus, 10 μL of the whole rye extract (10 g/15 mL) applied onto the HPTLC plate was allowed to contain 3.3 ng ergot alkaloids, which signal response was compared to the co-applied EC reference standard (10 μL, 333 pg/μL, 3.3 ng/band) as a benchmark. If the ergot alkaloid signal of the 10-μL whole rye extract was above the benchmark, the respective rye milling products were not marketable in the worst case, for example, for whole grain rye flour. The selected benchmark can be adjusted based on experience or on company internal limits. Since the LOQ of EC was 1.2 ng/zone, the benchmark can even be reduced to 180 μg/kg (instead of 500 μg/kg) for the given sample preparation.

Assuming that during modern mill technologies removing ergots, including transport processes, the whole grain is thoroughly mixed resulting in a homogeneous distribution of ergot dust contaminated grains. Therefore, sample sizes of only 10 g were selected also allowing low volumes of extraction solvents. The results obtained with both systems (Fig. 4) showed that none of the whole rye samples exceeded the selected benchmark visually and video-densitometrically (Instruction S3), although signals of traces of ergot alkaloids were detectable. The similar results and comparable validation data obtained by two independent analytical systems, *i.e.* the 2LabsToGo system *versus* the status quo HPTLC instrumentation, confirmed our hypothesis that the 2LabsToGo analysis is reliable and well suitable for performing screening for ergot alkaloids in whole rye. Method ruggedness was not the focus of this study. However, reliability regarding comparable results was given using two independent instrumental systems (Figs. 2 and 4) obtained by two different operators on different days and different instrumental parameters (Fig. S1) during the method development on both systems. The 2LabsToGo analysis was also suitable for cost-efficient, fast and simple screening, as explained in more detail below.

3.4. Advantages of instrumental miniaturization

Miniaturization, if it does not go too far (should still be able to be handled by the operator's hands), minimizes instrumental drawbacks and challenges. For instance, a smaller plate area facilitates uniform image illumination, while a streamlined instrument setup reduces errors and fits well within a lean and modest laboratory setup with all relevant steps nearby. Thereby, individual system parts have to fulfill several

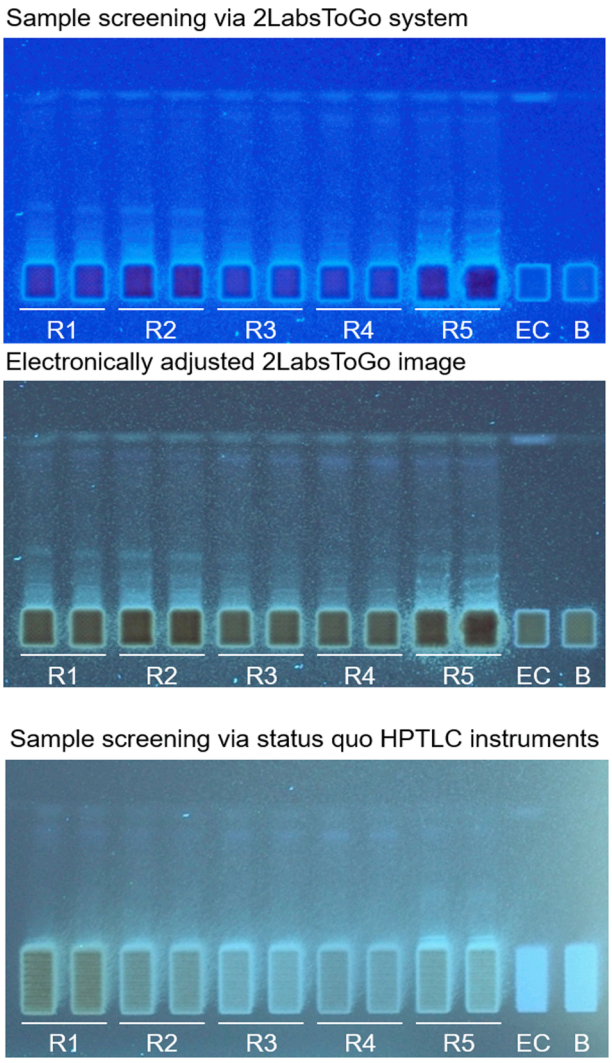

Fig. 4. Comparison of the screening of five different whole rye samples (R1–R5) for ergot alkaloids along with an EC standard solution (3.3 ng/area) and an extraction buffer blank (B), all applied as 10 μL and analyzed as in Fig. 2 via 2LabsToGo system (exemplarily, the blue background was electronically adjusted to black) *versus* conventional status quo HPTLC instruments (here 4 mm × 9 mm area). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

functionalities to keep the system footprint small. When comparing the calculation of instrumental costs, weight, and bench space, miniaturization proves to be advantageous (Fig. 1). Compared to status quo HPTLC instrumentation, the equipment costs were reduced by a factor of 40, the weight by a factor of 9, and the bench space requirement by a factor of 8. This has already been calculated in detail for the screening of saccharides in foods and lactose-free foods (Morlock et al., 2023) and applies here in the same way. The 2LabsToGo system has a low ecological footprint in terms of material consumption (sustainability goal of dematerialization). The equipment installed in one (chromatography laboratory) or two (plus biological assay laboratory, not demonstrated here) fully equipped laboratories is consolidated in a small-scaled all-in-one system. With only 7 kg weight and the minimal space requirements on the workbench, it supports high work efficiency (Lean Lab) and low electricity consumption. The portable and compact 2LabsToGo system can be operated at-site or even with solar panels in the field. It strikes the perfect balance in size and ease of handling towards sustainable analyses and can be considered as Lab of the Future, as it improved sustainability regarding method performance, equipment

resources, instrument maintenance and repair.

For example, in a recent ultra-high-performance liquid chromatography method (Huybrechts et al., 2021), samples were ground followed by a 60-min extraction and centrifugation and separated each with 5 mL mobile phase (10-min run with 0.5 mL/min), resulting in the consumption of 60 mL mobile phase for 12 samples. In comparison, 2LabsToGo analysis was comparatively rapid. Sample preparation took only 10 min for extraction and 20 min for centrifugation, while grinding was not necessary. The simultaneous chromatographic separation of 12 samples took 10 min (50 s/sample) and needed only 2 mL mobile phase, resulting in less than 0.2 mL mobile phase for one sample (25 times less solvent). Furthermore, evaluation of the 2LabsToGo method with the AGREE metrics software tool regarding sustainability (Pena-Pereira et al., 2020; Sajid & Płotka-Wasylka, 2022) has shown a mean scoring of 0.74 (Fig. S2), which indicated a good method greenness. In comparison, the ultra-high-performance liquid chromatography method by Huybrecht et al. (2021) reaches a score of 0.57 (Fig. S3).

The 2LabsToGo development is ongoing. In the latest 2LabsToGo system, a solar panel system has been integrated, enabling the system to operate independently from the power supply. Additionally, an open-source, cost-effective autosampler has been incorporated, increasing the grade of automation. The currently used separate raspistill scripts are not needed anymore, as Python bindings for the Raspberry Pi cameras were recently published elsewhere, enabling now long exposure times within the OCManager4 software. The image recording in the latest OCManager4 software has been adjusted to provide a dark chromatogram background instead of the currently blue one (Figs. 2-4). Sample application times and dynamic waiting times for development (Fig. S1) have been accelerated in the latest OCManager4 software and firmware increasing the current sample throughput.

4. Conclusions

The new 2LabsToGo technology stands out as a one-of-a-kind innovation in open-source system engineering of a sustainable Lean Lab of the Future. The screening of whole rye products for toxic ergot alkaloids was successfully demonstrated, evaluated and verified, expanding the proof-of-principle for the 2LabsToGo system and highlighting its feasibility for atline analysis in milling plants. This miniaturized all-in-one system delivered results on par with status quo HPTLC instruments but at significantly lower costs and lower consumption of materials and resources. Therefore, the 2LabsToGo system proves beneficial in situations where laboratory resources are scarce. It may inspire researchers to enhance and broaden the functionalities of the 2LabsToGo system for their own needs in various application fields. Particularly in detecting and prioritizing active compounds with biological effects, the 2LabsToGo system offers tailored solutions for future applications to address demanding analytical challenges. Via the biological detection on the same chromatogram, the non-target screening for previously unknown hazardous compounds in all kind of complex samples would improve consumer and environmental safety.

CRediT authorship contribution statement

Kevin Jakob: Writing – original draft, Validation, Methodology, Investigation, Formal analysis. **Wolfgang Schwack:** Writing – review & editing, Methodology, Conceptualization. **Gertrud E. Morlock:** Writing – review & editing, Writing – original draft, Visualization, Supervision, Resources, Methodology, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supplementary material

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References

Arcella, D., Gómez Ruiz, J.Á., Innocenti, M. L., & Roldán, R. (2017). Human and animal dietary exposure to ergot alkaloids. *EFSA Journal, 15*, 4902.

Bowd, A., Hudson, J. B., & Turnbull, J. H. (1973). Luminescence characteristics of lysergic acid diethylamide and related ergolines. *Journal of the Chemical Society, Perkin Transactions, 2*, 1312.

Bryła, M., Szymczyk, K., Jędrzejczak, R., & Roszko, M. (2015). Application of Liquid Chromatography/Ion Trap Mass Spectrometry Technique to Determine Ergot Alkaloids in Grain Products. *Food Technology and Biotechnology, 53*, 18–28.

Clarkson, E. D., Lesser, D., & Paul, B. D. (1998). Effective GC-MS procedure for detecting iso-LSD in urine after base-catalyzed conversion to LSD. *Clinical Chemistry, 44*, 287–292.

Commission of the European Communities. (2021). Commission Regulation (EU) 2021/1399 of 24 August 2021 amending Regulation (EC) No 1881/2006 as regards maximum levels of ergot sclerotia and ergot alkaloids in certain foodstuffs. *Office Journal of the European Union*, 1–5.

Di Diana Mavungu, J., Malysheva, S. V., Sanders, M., Larionova, D., Robbens, J., Dubruel, P., van Peteghem, C., & de Saeger, S. (2012). Development and validation of a new LC–MS/MS method for the simultaneous determination of six major ergot alkaloids and their corresponding epimers. Application to some food and feed commodities. *Food Chemistry, 135*, 292–303.

EFSA Panel on Contaminants in the Food Chain (CONTAM). (2012). Scientific Opinion on Ergot alkaloids in food and feed. *EFSA Journal, 10*, 2798.

Fichou, D., & Morlock, G. E. (2018). quanTLC, an online open-source solution for videodensitometric quantification. *Journal of Chromatography. A, 1560*, 78–81.

Flieger, M., Wurst, M., & Shelby, R. (1997). Ergot alkaloids—sources, structures and analytical methods. *Folia Microbiologica, 42*, 3–29.

Franzmann, C., Schröder, J., Münzing, K., Wolf, K., Lindhauer, M. G., & Humpf, H.-U. (2011). Distribution of ergot alkaloids and ricinoleic acid in different milling fractions. *Mycotoxin Research, 27*, 13–21.

Friedman, A. P., von Storch, T. J., & Araki, S. (1959). Ergotamine tartrate: Its history, action, and proper use in the treatment of migraine. *New York State Journal of Medicine, 59*, 2359–2366.

de Groot, A. N., van Dongen, P. W., Vree, T. B., Hekster, Y. A., & van Roosmalen, J. (1998). Ergot alkaloids. Current status and review of clinical pharmacology and therapeutic use compared with other oxytocics in obstetrics and gynaecology. *Drugs, 56*, 523–535.

Haarmann, T., Rolke, Y., Giesbert, S., & Tudzynski, P. (2009). Ergot: From witchcraft to biotechnology. *Molecular Plant Pathology, 10*, 563–577.

Huybrechts, B., Malysheva, S. V., & Masquelier, J. (2021). A Targeted UHPLC-MS/MS Method Validated for the Quantification of Ergot Alkaloids in Cereal-Based Baby Food from the Belgian Market. *Toxins, 13*, 531.

ICH (1995/2005). *Q 2(R1): Validation of Analytical Procedures: Text and Methodology*: International Council for Harmonisation.

Köppen, R., Rasenko, T., Merkel, S., Mönch, B., & Koch, M. (2013). Novel solid-phase extraction for epimer-specific quantitation of ergot alkaloids in rye flour and wheat germ oil. *Journal of Agricultural and Food Chemistry, 61*, 10699–10707.

Krska, R., & Crews, C. (2008). Significance, chemistry and determination of ergot alkaloids: A review. *Food Additives & Contaminants. Part A, 25*, 722–731.

Krska, R., Stubbings, G., Macarthur, R., & Crews, C. (2008). Simultaneous determination of six major ergot alkaloids and their epimers in cereals and foodstuffs by LC-MS-MS. *Analytical and Bioanalytical Chemistry, 391*, 563–576.

Lorenz, K. (1979). Ergot on cereal grains. *CRC Critical Reviews in Food Science and Nutrition, 11*, 311–354.

Mohamed, R., Gremaud, E., Richoz-Payot, J., Tabet, J.-C., & Guy, P. A. (2006). Quantitative determination of five ergot alkaloids in rye flour by liquid

chromatography-electrospray ionisation tandem mass spectrometry. *Journal of Chromatography A, 1114*, 62–72.
Morlock, G. E., Koch, J., & Schwack, W. (2023). Miniaturized open-source 2LabsToGo screening of lactose-free dairy products and saccharide-containing foods. *Journal of Chromatography A, 1688*, Article 463720.
Oellig, C., & Melde, T. (2016). Screening for total ergot alkaloids in rye flour by planar solid phase extraction-fluorescence detection and mass spectrometry. *Journal of Chromatography A, 1441*, 126–133.
Pena-Pereira, F., Wojnowski, W., & Tobiszewski, M. (2020). AGREE-Analytical GREEnness Metric Approach and Software. *Analytical chemistry, 92*, 10076–10082.
Richard, J. L. (2007). Some major mycotoxins and their mycotoxicoses—an overview. *International Journal of Food Microbiology, 119*, 3–10.
Sajid, M., & Płotka-Wasylka, J. (2022). Green analytical chemistry metrics: A review. *Talanta, 238*, Article 123046.
Schade, F., Schwack, W., Demirbas, Y., & Morlock, G. E. (2021). Open-source all-in-one LabToGo Office Chromatography. *Analytica Chimica Acta, 1174*, Article 338702.
Scott, P. M. (1993). Gas Chromatography of Mycotoxins. In V. Betina (Ed.), *Chromatography of Mycotoxins* (pp. 373–425). Amsterdam: Elsevier.
Scott, P. M., & Lawrence, G. A. (1980). Analysis of ergot alkaloids in flour. *Journal of Agricultural and Food Chemistry, 28*, 1258–1261.
Sing, L., Schwack, W., Göttsche, R., & Morlock, G. E. (2022). 2LabsToGo—Recipe for Building Your Own Chromatography Equipment Including Biological Assay and Effect Detection. *Analytical Chemistry, 94*, 14554–14564.
Storm, I. D., Rasmussen, P. H., Strobel, B. W., & Hansen, H. C. B. (2008). Ergot alkaloids in rye flour determined by solid-phase cation-exchange and high-pressure liquid chromatography with fluorescence detection. *Food Additives & Contaminants. Part A, 25*, 338–346.
Sharma, N., Sharma, V. K., Kumar Manikyam, H., & Bal Krishna, A. (2016). Ergot Alkaloids: A Review on Therapeutic Applications. *European Journal of Medicinal Plants, 14*, 1–17.