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Confirmatory Analysis of Ergot Alkaloids in Rye Flour by Liquid Chromatography coupled to Tandem Mass Spectrometry*

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Introduction

Ergot is the name given to the *sclerotium* of the *fungus Claviceps* species that infect cereals. These ergots produce a range of up to 40 different alkaloids. They have been shown to induce hallucinations, agitation and other symptoms in humans. All the common cereals including rye, wheat, barley, millet and maize can be infected with ergot alkaloids, although rye is the most susceptible one. Up to now, no legislation has been reported by the European Union towards these chemicals, but several countries, including Switzerland have set their own limits for the presence of ergot alkaloids at 500 mg/kg in cereal grain for flour trade and at 200 mg/kg in cereal grains for direct consumption (limit refers to the weight of ergot kernels per total commodity weight, and not to toxin concentration). All the common analytical techniques such as thin layer chromatography (TLC) (1), high performance liquid chromatography (HPLC) (2), gas liquid chromatography system (GLC) (3) and gas chromatography coupled with mass spectrometry (GC-MS) (4) have been used for the determination of ergot alkaloids and lysergic acid derivatives. Several matrices were studied such as cereals and cereals products (5), human and animal biological fluids (6). To provide an unambiguous monitoring of such mycotoxins, we have developed an HPLC-MS/MS method for the quantitative determi-

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nation of ergonovine, ergotamine, ergocristine, α-ergokryptine and ergocornine residues in rye flour samples.

Methods

Methysergide hydrogen maleinate (MHM) (kind gift from Sandoz, Schonewerd, CH) was spiked in rye flour samples to assess both reproducible recovery and good LC-MS/MS operation over time. Ergonovine, ergotamine, ergocristine, α-ergocryptine and ergocornine (Buchs, CH) were quantified in rye flour using external calibration curve of an 8-data points.

An aliquot of rye flour (5 g) is weighed in a Teflon centrifugation tube, solubilised in 10 mL of acetonitrile and 20 mL of ammonium acetate (10 mM, pH 6.5). The mixture is then stirred horizontally for 20 minutes before being centrifuged at 10000 rpm for 10 minutes (4 °C). A 2 mL aliquot of the supernatant is loaded onto the Chromabond C18 ec SPE cartridge, previously conditioned with successively 2 ml of methanol and 2 ml of 10 mM ammonium acetate (pH 6.5). After loading, the extract is washed with 2 mL of water/acetonitrile (9:1, v/v). The elution step is performed with 2 mL of methanol/acetonitrile (1:1, v/v) and the extract evaporated at 40 °C under a light stream of nitrogen. Dry residue was reconstituted in 2 mL of 5 mM heptafluoroburytic acid (HFBA)/acetonitrile (9:1, v/v) and filtered onto a 0.2 µm nylon filter (Nalgene, Rochester, NY, USA) directly into an HPLC vial.

Separation of the analytes was performed on a Perkin Elmer pump Series 200 HPLC system (Perkin Elmer, Germany). The HPLC column was a X-Terra C18 reversed phase (2.1 mm×100 mm, 3.5 µm) and the mobile phase constituted of solvent A: 5mM HFBA and solvent B: acetonitrile containing 0.1 % acetic acid. The gradient program was: 0–2 min 10 % B; 2–12 min 65 % B; 12–13 min 65 % B; 13–13.1 min 90 % B; 13.1–19.1 min 90 % B; 19.1–20 min 10% B and 20–25 min 10 % B running at a constant flow rate of 0.3 mL/min. The injection volume was 10 µL.

Ergot alkaloids were detected on an API 3000 TurboIonSpray (Applied Biosystems, USA) triple stage quadrupole mass spectrometer in positive electrospray ionisation using selected reaction monitoring (SRM) acquisition mode. The quantitative analysis was performed using the most intense signal whereas the second was used for analyte confirmation based on appropriate area ratio calculated from standards. A common intense ion at *m/z* 223 is revealed for all the ergot alkaloids studied (figure 1), therefore the analysis in precursor ion mode was also evaluated to find potential others ergots alkaloids (lysergic acid and peptide type) in unknown samples. External calibration curve was constructed from a concentration range of 0 to 25 ng (10 ng of MHM) injected on-column, corresponding to a contamination levels ranging from 0–15 mg/kg.

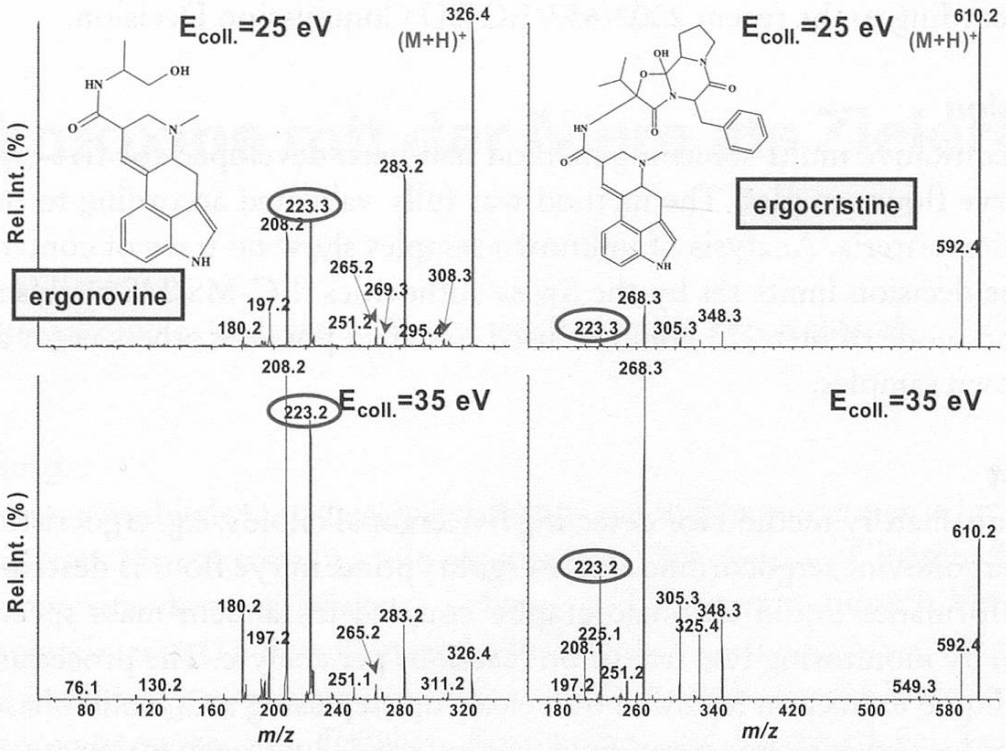

Figure 1 **CID mass spectra of ergonovine and ergocristine realized at collision energies of 25 and 35 eV**

Results

A basic sample preparation was targeted to enable a higher sample throughput. Therefore, a liquid-liquid extraction was evaluated before LC-MS/MS analysis but due to a relatively high matrix effect, linked with co-eluting chemical interferences, we decided to add a clean-up step using a C18 end-capped solid phase extraction cartridge. Using such sample preparation, some recovery experiments were realized by comparing the areas obtained from blank rye flour sample spiked (independent triplicates at concentration levels of 3, 7.5 and 15 mg/kg) at the right beginning versus those from spiked extracts prior to LC-MS/MS analysis. Overall, the recoveries were above 24 % for all ergot alkaloids.

The ergot alkaloids are well separated on a X-Terra C18 column in a 13 min run time. A possible matrix effect, described for the use of LC-MS/MS technique, was evaluated by comparing the blank rye flour extracts spiked prior to analysis with same amount of analyte injected from standard solutions.

The confirmation of the presence of ergot alkaloids in unknown rye flour was made when identical retention times were obtained compared with those on standard solutions. Moreover, the area ratio of the two SRM monitored for each analyte needed to be within an appropriate range obtained from the analysis of standard solutions. Once the presence of an analyte was confirmed, its quantification was done from the most intense SRM and data generated were compared with external

calibration curves made in spiked blank matrix. The whole method was fully validated according to the recent 2002/657/EC EU Commission Decision.

Conclusion

A quantitative multi-screening method had been developed for five ergot alkaloids in rye flour samples. The method was fully validated according to the European Union criteria. Analysis of unknown samples show no trace of contamination above the decision limits set by the Swiss authorities. LC-MS/MS analysis in precursor ion mode of *m/z* 223 could be used to detect possible others ergot alkaloids in unknown samples.

Abstract

A confirmatory method for detecting five ergot alkaloids, e.g. ergocristine, ergotamine, ergonovine, ergocornine and α-ergokryptine, in rye flour is described using high performance liquid chromatography coupled to tandem mass spectrometry detection by monitoring two transition reactions per analyte. The procedure entails a liquid-liquid extraction followed by a clean-up step using a C_{18} solid phase extraction cartridge. An analogue compound, methysergide hydrogen maleinate, was used to assess both reproducible sample preparation and potential MS response fluctuations. The method was fully validated according to the European Union (EU) criteria. A common intense ion at *m/z* 223 had been identified and could enable the detection of traces of ergot alkaloids in unknown samples.

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