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Quantitative determination of five ergot alkaloids in rye flour by liquid chromatography–electrospray ionisation tandem mass spectrometry

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Abstract

A confirmatory method for detecting five ergot alkaloids, 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 C18 solid-phase extraction (SPE) cartridge. An analogue compound, methysergide hydrogen maleinate, was used to assess both repeatability sample preparation and potential MS response fluctuations. The method was fully validated according to the European Union (EU) criteria. Detection and quantification limits of all analytes were calculated ranging from 7 to 11 μg/kg and from 23 to 37 μg/kg, respectively. Fifteen rye flour samples were investigated with the newly developed method, and none of them were above the current Swiss limits of 200 mg/kg for total ergot alkaloids.

Keywords: Ergot alkaloids; Rye flour; Liquid chromatography tandem mass spectrometry; Quantification; Analyte confirmation

1. Introduction

Ergot is the name given to the *sclerotium* of the fungus *Claviceps purpurea* and some other Claviceps species that infect many wild grasses and cereals [1]. These hard black tuber-like bodies consist of a compact mass of hyphae and are the resting stage of the fungus. These ergots produce a range of up to 40 different alkaloids that can lead to the formation of lysergic acid if the ergot ferments. Lysergic acid causes hallucinations, agitation and other symptoms [2]. In Europe, from the 8th to 16th centuries, ergot in rye consumed by the population was the main cause of Holy fire, '*ignis sacer*', or St. Anthony's fire; and the effects included gangrenous ergotism, burning sensations or hallucinations [3]. Ergot was first recognised as a fungus in the 18th century and known as a source of drugs by the beginning of the 19th century. The main ergot alkaloids are ergotamine, ergocornine, ergocristine, ergokryptine, ergometrine and agroclavine. These chemicals can be differentiated as water-soluble lysergic acid derivatives and water-insoluble peptide-type ergot alkaloids (Fig. 1). All the common cereals including rye, wheat, barley, triticale, oats, millet, sorghum and maize can be infected with ergot, although rye has been reported as the most susceptible one [4]. In Europe, rye bread has often been linked to outbreaks of ergotism [5]. The European Union (EU) has defined a limit for the presence of ergot alkaloids in wheat at 0.05 wt.% [6] and several countries, including Switzerland, have set their own limits for the presence of total ergot alkaloids at 200 mg/kg in cereals grains for direct consumption [7].

All the common analytical techniques such as thin layer chromatography (TLC) [8,9], high-performance liquid chromatography (HPLC) coupled with ultra-violet detection [10,11], gas–liquid chromatography system (GLC) with electron capture detection [12,13] and gas chromatography with mass spectrometry detection (GC–MS) [14,15] have been reported for the determination of ergot alkaloids and lysergic acid deriva-

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tives. Several matrices were studied such as cereals and cereals products [14,16,17], and human and animal biological fluids [9,10,13,15,18]. TLC was developed using a wide range of solvent systems to separate such analytes either on silica gel, alumina, or formamide-impregnated cellulose layers. However, this technique suffers from a poor separation power in some cases (i.e. ergocornine, ergocristine and ergokryptine). Few publications involving GC–MS techniques were developed but several drawbacks were identified essentially due to the detection issues encountered during the analysis of such analytes in terms of: (i) relatively high molecular weight (up to 609 u); (ii) low vapour pressure and (iii) instability towards heating. In contrast, HPLC coupled to ultra-violet and fluorometric detections [19,20] were widely reported mainly using reverse-phase C18 columns with alkaline mobile phases containing ammonium carbonate, triethylamine or acetonitrile-phosphate buffered (pH 7). Alternatively, HPLC coupled to electrospray ionisation mass spectrometry (LC–ESI/MS) detection has provided an unequivocal identification of the alkaloids [18,21].

In the present work, we describe a confirmatory and quantitative HPLC method coupled to tandem mass spectrometry for the determination of five ergot alkaloids (i.e. ergonovine, MW = 325 u; ergotamine, MW = 581 u; ergocornine, MW = 561 u; α-ergokryptine, MW = 575 u and ergocristine, MW = 609 u; Fig. 1) in rye flour samples. Our method was fully validated according to the EU guidelines regarding the analytical performance of spectrometry techniques [22]. To our knowledge, no such multi-screening mass spectrometry based method has been previously reported in cereal matrices.

2. Materials and methods

2.1. Chemicals and reagents

Ergonovine tartrate, ergocornine, α-ergokryptine, ergotamine and ergocristine were obtained from Sigma (Buchs, Switzerland). Methysergide hydrogen maleinate (MHM) was a kindly gift from Sandoz (Schonenwerd, Switzerland). Acetonitrile and methanol (gradient grade for liquid chromatography) were supplied by Merck (Dietikon, Switzerland). Heptafluorobutyric acid (HFBA) was purchased from Aldrich (Buchs, Switzerland). The Chromabond C18 end capped (ec) (500 mg, 3 mL) solid-phase extraction (SPE) cartridge was purchased from Macherey-Nagel (Oensingen, Switzerland). Deionised and distilled water was obtained from a Milli-Q water purification apparatus (Millipore, Bedford, MA, USA).

2.2. Standard solutions

Stock solutions of each analyte were prepared individually at a concentration of 1 mg/ml in methanol (for α-ergokryptine, ergonovine, ergotamine and MHM) and in acetonitrile (for ergocornine and ergocristine); and stored at −20 °C in darkness for a week to prevent any isomerisation problems already reported for these chemicals [23–25]. Diluted standard solutions were freshly prepared in water before use.

2.3. Rye flour samples

Different types of rye flour/flake samples were purchased from local Swiss supermarkets. Samples were aliquoted and

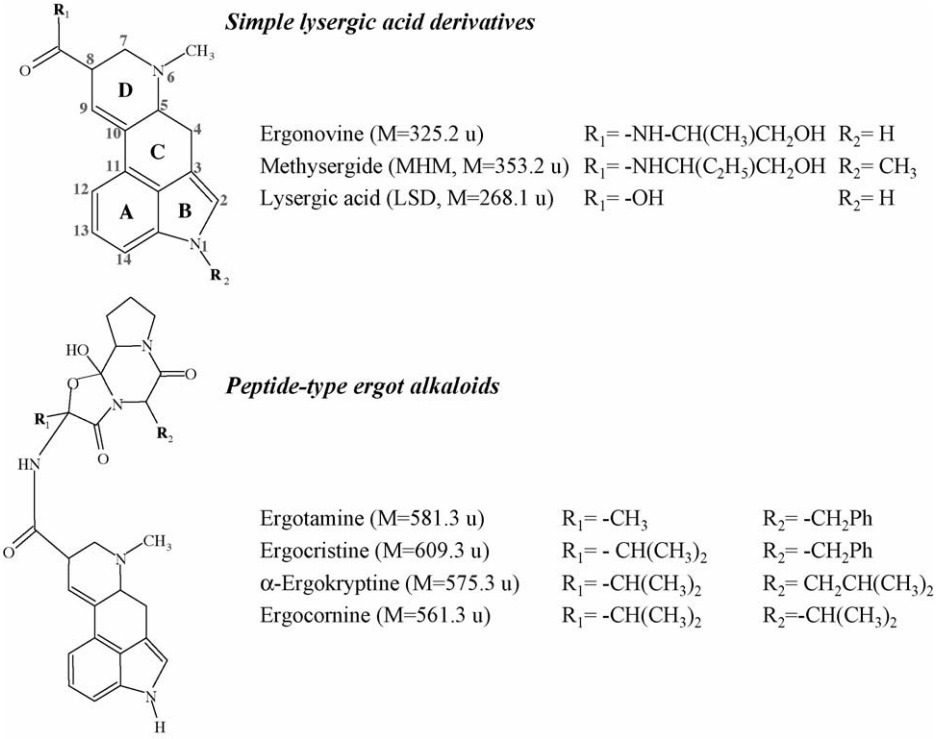

Fig. 1. Chemical structures of the simple lysergic acid derivatives and peptide-type ergot alkaloids.