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Separation of ergot alkaloids and their epimers and determination in sclerotia by capillary electrophoresis

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Abstract

Capillary electrophoresis has been shown to be a very useful analysis technique for secondary metabolites of plants. In the present study a method is described for the qualitative and quantitative determination of ergot alkaloids and their epimers. The extraction from the biological matrix yields recoveries of 50–97%, depending on the individual alkaloid. Using a mixture of 20 m*M* β-cyclodextrin (CD), 8 m*M* γ-CD, 2 *M* urea and 0.3% (w/w) poly(vinyl alcohol) to phosphate buffer at pH 2.5 the simultaneous separation of all analytes was achieved. A 37 cm (30 cm) fused-silica capillary, at a voltage of 25 kV and a temperature of 20°C, was used for the analysis. Overall analysis time for the separation was 12 min. The limit of detection of the alkaloids using UV detection at 214 nm can be improved 30-fold to about $9 \cdot 10^{-8}$ *M* when laser-induced fluorescence detection is applied. © 1998 Elsevier Science B.V. All rights reserved.

Keywords: Rye; Alkaloids

1. Introduction

Ergot, the sclerotia of the fungus *Claviceps purpurea*, is found as a parasite on more than 400 genera of the poaceae of which rye is of particular concern.

Ergot alkaloids contain the tetracyclic ergoline ring system, which is a partially hydrogenated quinoline containing a carboxylic acid group in the 8-position. The alkaloids are divided into two groups. Lysergic acid is either connected to a amino alcohol side chain (Fig. 1, alkaloids 1–2) or linked to a tricyclic peptide by a peptide bond (Fig. 1, alkaloids 3–9).

Under the influence of acid or base, ergot alkaloids undergo isomerisation in position 8. The physiologically active (5*R*,8*R*)-lysergic acid derivates are converted to inactive (5*R*,8*S*)-isolysergic acid epimers [1] e.g., ergonovine into ergonovinine or ergocornine into ergocorninine.

The double bond in position 9,10 has been shown as a prerequisite for the native fluorescence, offering the possibility of fluorescence detection. This has been applied to high-performance liquid chromatographic analysis [2–5] of genuine ergot alkaloids.

In this paper a capillary electrophoresis (CE) method was developed for the determination of ergonovinine (1), ergonovine (2), ergocorninine (3),

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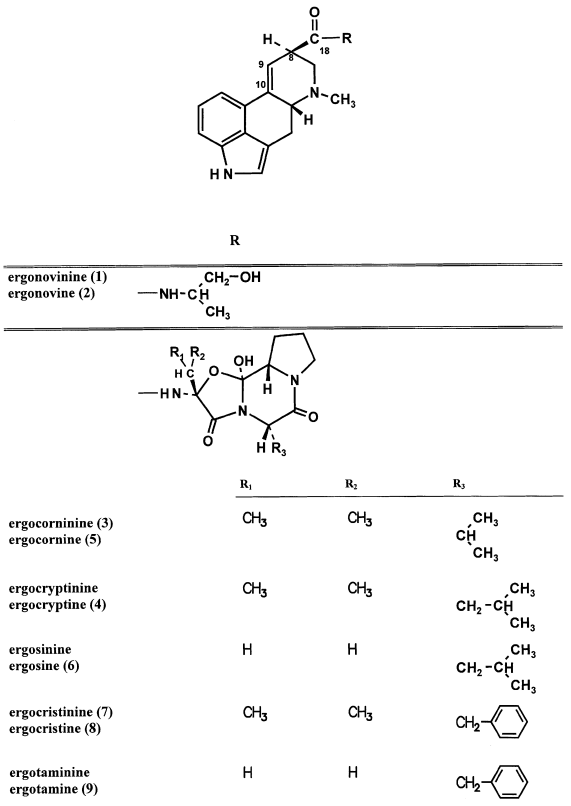

Fig. 1. Structure of ergot alkaloids

ergocryptine (4), ergocornine (5), ergosine (6), ergocristinine (7), ergocristine (8) and ergotamine (9) (Fig. 1).

2. Experimental

2.1. Reagents and chemicals

Ergonovinine, ergocryptine, ergocornine, ergosine and ergocristinine were gifts from Dr. Ing. J. Wolff (Bundesanstalt für Getreide-, Kartoffel- und Fettforschung, Detmold, Germany). Ergonovine and ergotamine were donated by Sandoz Pharma (Basel, Switzerland). Boehringer Ingelheim (Ingelheim, Germany) gave a contribution of ergocorninine and ergocristine. Lisuride was a gift from Schering (Berlin, Germany) and used as the internal standard. Standard solutions were prepared in methanol–10 m*M* hydrochloric acid (1:1). Solutions were freshly made before each new set of runs.

Orthophosphoric acid (<85%), hydrochloric acid, sodium dihydrogenphosphate and urea were purchased from Fluka (Deisenhofen, Germany). Native β- and γ-cyclodextrins (CDs) were obtained from Wacker Chemie (Burghausen, Germany), poly(vinyl alcohol) (PVA) from Aldrich (Steinheim, Germany). Ethyl acetate, dichloromethane, methanol and ammonium hydroxide (25%) (Baker, Griesheim, Germany) were of analytical grade.

2.2. Extraction and sample preparation

One hundred μg of crushed ergot of rye were extracted with 0.9 ml dichloromethane–ethyl acetate–methanol–25% ammonium hydroxide (25:12.5:2.5:0.5) [6,7] and 0.1 ml solution of internal standard (0.4 mg lisuride/1.00 ml methanol–10.00 m*M* hydrochloric acid, 1:1) was added. The ergot extraction mixture was vortex mixed and centrifuged for 10 min at 3500 *g* at room temperature. The supernatant was separated. This extraction was repeated two times. The supernatants were combined and the solvents were removed under a gentle stream of nitrogen.

The residue was reconstituted in 1.00 ml 10 m*M* hydrochloric acid–methanol (1:1) and filtered through a 0.2 nylon filter (Carl Roth, Karlsruhe, Germany) prior to the injection into the CE system. The sample was hydrodynamically injected for 2 s at 0.5 p.s.i. (1 p.s.i.=6894.76 Pa).

2.3. Equipment

All analyses were performed on a P/ACE 2200 CE system (Beckman, Munich, Germany). Two kinds of detectors were used, a UV detector (detection wavelength: 214 nm) and a He–Cd laser (Model 3074-EOS-A01, 20 mW, Omnichrome, Bischofswerda, Germany) using an excitation wavelength of 325 nm and an emission wavelength of 436 nm.

2.4. Electrophoretic conditions for the assay

The capillary had a total length of 37 cm (30 cm to detector) and an inner diameter of 50 μm. The outer diameter was 360 μm. The temperature of the

capillary was kept at 20°C and the voltage applied was 25 kV.

The standard run buffer consisted of an aqueous solution of 50 m*M* sodium dihydrogenphosphate, adjusted to pH 2.5 with 50 m*M* phosphoric acid, containing 20 m*M* β-CD, 8 m*M* γ-CD, 2 *M* urea and 0.3% (w/w) PVA.

Before sample injection the capillary was rinsed 2 min with 250 m*M* phosphoric acid and 2 min with run buffer. The detection was towards the cathodic end.

2.5. Calibration

As no blank matrix of sclerotia exist, crushed sclerotia was extracted as described above until no alkaloid was detectable. There were still enough matrix substances which interfered with the alkaloids during separation. This could be proved by a direct comparison between standard solution and spiked blank matrix.

Calibration curves were calculated by adding variable quantities of the standard solution of the alkaloids to the extracted ergot matrix. The samples were processed as described in Section 2.2 beginning with the first extraction with alkaline organic solvent and the addition of the internal standard. The plots of the linear curves were obtained by plotting concentration versus peak area ratios of the alkaloids and the internal standard.

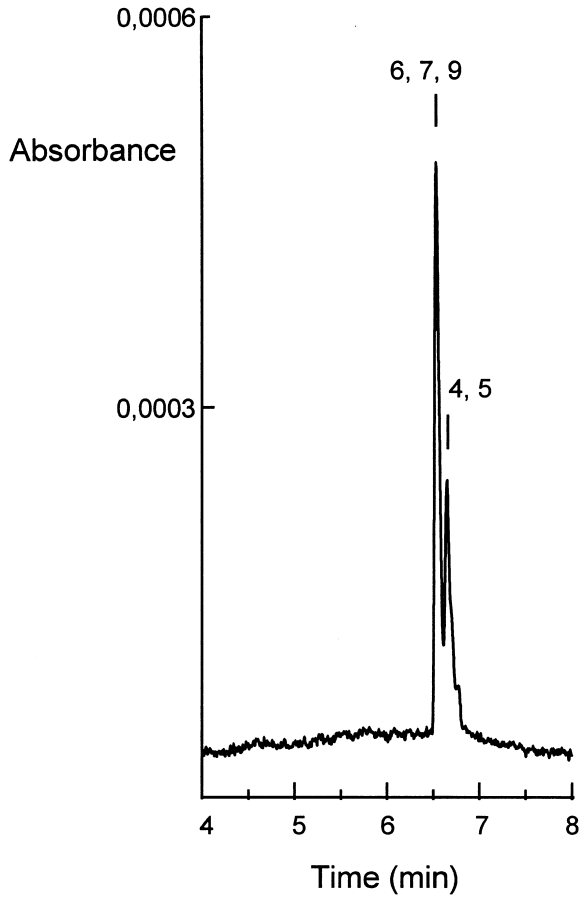

Fig. 2. Electropherogram of a standard alkaloid mixture. Conditions: 50 m*M* phosphate buffer, pH 2.5; 4=ergocryptine, 5=ergocornine, 6=ergosine, 7=ergocristinine, 9=ergotamine; fused-silica capillary: 37 cm (30 cm)×50 μm I.D.; UV detection at 214 nm.

3. Results

3.1. Development of the method

With the use of 50 m*M* phosphate buffer, pH 2.5 no separation of the alkaloids was obtained (Fig. 2). The addition of native β- or γ-CDs led to a partial separation of five alkaloids with a comigration of two different pairs of alkaloids. Using β-CD ergonovine and ergonovinine as well as ergocryptine and ergocornine comigrated as shown in Fig. 3. When γ-CD was employed ergocryptine and ergosine as well as ergocornine and ergocristinine comigrated (data not shown).

Optimization of the CD ratio finally led to a combination of 20 m*M* β-CD and 8 m*M* γ-CD. In order to improve the solubility of the CDs urea was added [8]. This additive optimized the resolution. PVA as an additive increased the selectivity of all analytes resulting in a complete separation of the nine compounds shown in Fig. 4. The buffer had to be changed after 10 runs.

Due to interactions of the ergot alkaloids with both CDs, complexes with different stability were formed which led to different effective mobilities. This effect has been previously observed not only for enantiomers but also for mixtures of different components [9,10].

In comparison to high-performance liquid chromatography (HPLC) a simplified and faster extraction

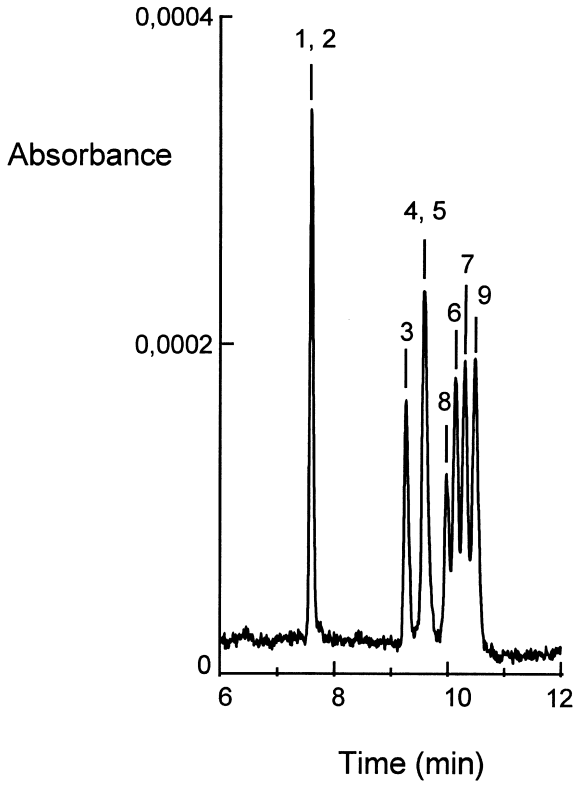

Fig. 3. Electropherogram of a standard alkaloid mixture. Conditions: 50 m*M* phosphate buffer, pH 2.5 and 15 m*M* β-CD; 1=ergonovinine, 2=ergonovine, 3=ergocorninine, 4=ergocryptine, 5=ergocornine, 6=ergosine, 7=ergocristinine, 8=ergocristine, 9=ergotamine; for other conditions see Fig. 2.

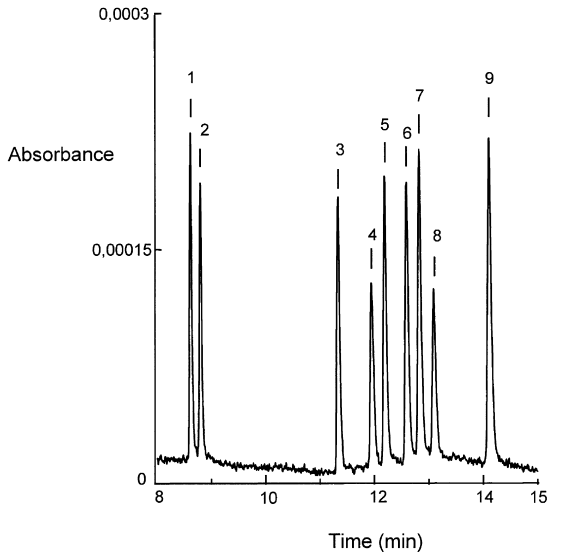

Fig. 4. Electropherogram of a standard alkaloid mixture. Conditions: standard buffer [20 m*M* β-CD, 8 m*M* γ-CD, 2 *M* urea, 0.3% (w/w) PVA]; 1=ergonovinine, 2=ergonovine, 3=ergocorninine, 4=ergocryptine, 5=ergocornine, 6=ergosine, 7=ergocristinine, 8=ergocristin, 9=ergotamine; for other conditions see Fig. 2.

method and shorter analysis time were obtained. For determination of ergot alkaloids in cereals some authors required 25 min by HPLC [11] and more than 50 min [12], respectively.

3.2. Assay procedure

In these preliminary experiments a UV detector was used. For the investigation of the sclerotia laser-induced fluorescence (LIF) detection using a He–Cd laser was applied. The sensitivity was about 30-times higher compared with UV detection. Fig. 5 shows the electropherogram of the same mixture of seven alkaloids obtained by UV (A) and LIF (B) detection.

Linearity, precision and recovery were determined for each alkaloid. For example the calibration curve of ergotamine was constructed using a concentration of 5–30 μg alkaloid/ml solvent. By linear regression analysis of the concentration versus peak area ratios,

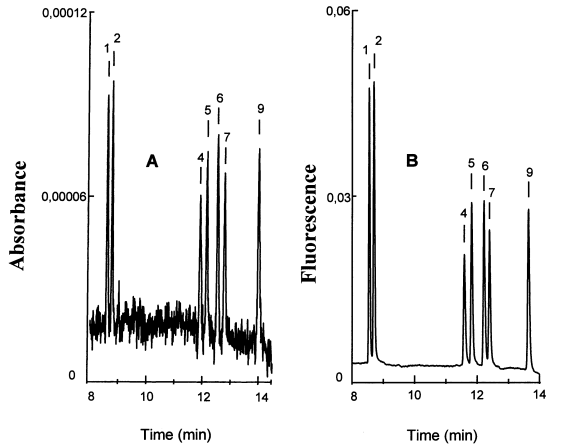

Fig. 5. Electropherograms showing a comparison whith UV (A) and LIF (B) detection of the CE analysis of an alkaloid mixture. Conditions: standard buffer; 1=ergonovinine, 2=ergonovine, 4=ergocryptine, 5=ergocornine, 6=ergosine, 7=ergocristinine, 9=ergotamine; for conditions see Fig. 4.

coefficients of higher than 0.9985 were calculated. Recovery was determined by comparison of peak areas of extracted alkaloid standards with the peak area obtained from direct injection of standards of equivalent concentrations dissolved in hydrochloric acid–methanol. For ergotamine the rate of recovery was 93.7%. Table 1 shows the precision data for ergotamine. The relative standard deviation (R.S.D.) for inter-assay is <3% throughout the entire range of calibration.

Inter-assay precision for the other alkaloids were between 2.9 and 15.6%, R.S.D. and the recoveries between 60.9% for ergocornine and 96.6% for ergonovinine.

3.3. Applications

Alkaloids of the sclerotia slowly decompose and isomerise to isolysergic acid derivates. They can be expected to be stable for only about 1 year [13]. In sclerotia which were 20–25 years old alkaloids are still detectable (Fig. 6). Furthermore, different sclerotia of rye were analyzed. The results are summarized in Table 2. The content of alkaloids varied between 0.02% (w/v) and 45.9% (w/v) for cultivated sclerotia depending on the origin of the sample.

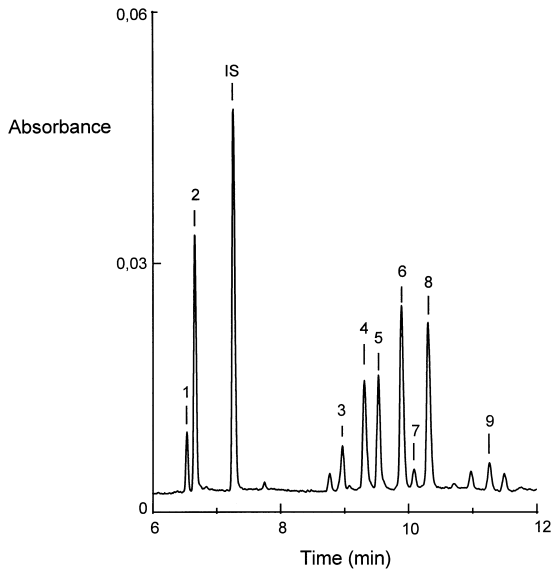

Fig. 6. CE analysis of 20–25-year-old cultivated sclerotia. Conditions: standard buffer; 1=ergonovinine, 2=ergonovine, I.S.=lisuride, 3=ergocorninine, 4=ergocryptine, 5=ergocornine, 6=ergosine, 7=ergocristinine, 8=ergocristin, 9=ergotamine; for conditions see Fig. 4.

4. Conclusion

The proposed method provides a rapid and sensitive determination of ergot alkaloids. The application

Table 1
Precision and accuracy of the determination of ergotamine in sclerotia matrix with CE–LIF

	Concentration added (μg/ml)				
	5.0	7.5	15.0	22.5	30.0
Concentration found (μg/ml) arith. mean value					
Series 1 (n=5)	4.9	7.6	14.7	21.9	29.4
Series 2 (n=5)	4.4	7.4	14.9	21.6	30.9
Series 3 (n=5)	4.6	7.4	15.3	23.2	31.4
Inter-assay	4.6	7.5	15.0	22.3	30.6
Precision (R.S.D., %) arith. mean value					
Series 1 (n=5)	2.0	0.9	1.3	0.5	1.9
Series 2 (n=5)	4.5	2.7	1.0	0.6	1.0
Series 3 (n=5)	2.1	1.6	2.2	1.6	0.9
Inter-assay	4.5	1.4	1.7	3.0	2.7
Accuracy (%) arith. mean value					
Series 1 (n=5)	97.2	101.7	98.1	97.5	98.0
Series 2 (n=5)	87.2	98.5	99.4	96.1	102.8
Series 3 (n=5)	91.9	99.0	102.2	103.1	104.6

Table 2
Concentration (μg/ml) of ergot alkaloids in different sclerotia

	Ergonovinine	Ergonovine	Ergocorninine	Ergocryptine	Ergocornine
MS	0.2	2.8	–	3.3	–
GS	–	1.5	–	–	–
FS	3.2	24.2	6.3	31.3	29.8
OS	5.2	25.0	5.5	31.5	18.1
BIS	10.9	45.8	14.4	92.6	5.0
	Ergosine	Ergocristinine	Ergotaminine	Ergocristine	Ergotamine
Ms	3.9	–	–	1.5	0.9
GS	–	3.4	–	36.5	4.7
FS	33.7	2.6	4.1	9.0	2.95
OS	33.7	2.5	–	21.6	2.7
BIS	14.8	61.0	–	459.2	6.0

MS=Sclerotia from rye grown close to Münster (western part of Germany).
GS=Gene manipulated sclerotia from the Institute of Botany, University of Münster.
FS=Rank grown sclerotia from Franken (southern part of Germany).
OS=20–25-year-old cultivated sclerotia.
BIS=Ergocristine-containing sclerotia from Boehringer Ingelheim.

of LIF using a He–Cd laser increases the selectivity and sensitivity. The main alkaloids from different sclerotia can be easily analysed very sensitively.

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