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Quantification of *Fumaria officinalis* isoquinoline alkaloids by nonaqueous capillary electrophoresis–electrospray ion trap mass spectrometry

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Abstract

A capillary electrophoresis (CE) method using non-aqueous (NA) separation solutions combined with an ion trap mass spectrometer (MS and MS/MS) as detection device is presented for the separation, identification and quantification of isoquinoline alkaloids from *Fumaria officinalis*. The best results were obtained with a mixture of acetonitrile–methanol (9:1, v/v) containing 60 mM ammonium acetate and 2.2 M acetic acid as running electrolyte and an applied voltage of 30 kV. Electrospray MS measurements were performed in the positive ionization mode with isopropanol–water (1:1, v/v) as sheath liquid at a flow rate of 3 μl/min. Alkaloids were detected as $[M+H]^+$-ions and showed typical fragmentation patterns in MS/MS experiments. The developed assay was used for the quantification of seven isoquinoline alkaloids representing different structural subtypes in *Fumariae herba* extracts and *F. herba* containing phytopharmaceuticals.

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Keywords: *Fumaria officinalis*; Isoquinoline alkaloids; Non-aqueous capillary electrophoresis; On-line electrospray ion trap mass spectrometry

1. Introduction

Fumaria officinalis L. (Fumariaceae) is a widespread perennial herb in Europe and Asia which has played a traditional role in empirical medicine over centuries. Fumitory or earth smoke has been used in many countries for the treatment of skin diseases, rheumatism, hypertension or infections. Nowadays, this herb is a component in several phytopharmaceuticals, mainly used to treat functional diseases of the hepatobiliary system discernible as colicky pains affecting the gallbladder, biliary system and the gastrointestinal tract. These biological activities of *Fumariae herba* are mainly associated with the presence of isoquinoline alkaloids [1,2].

However, qualitative and quantitative analytical assays of this structure class are often hampered by the charged character of the tertiary bases. Therefore, only a quite limited number of TLC, GC–MS [3], HPLC [4] and isotachophoresis [5,6] based methods have been established for the analysis of *Fumaria* alkaloids.

Capillary electrophoresis (CE) offers a number of methodological benefits, such as high resolution capability due to the flat flow profile, low sample and solvent consumption, and inexpensive replacement of capillaries [7]. The use of non-aqueous (NA) media in CE represents an interesting alternative to buffer solutions in water. Compared to aqueous solutions, several physical and chemical properties of organic solvents, such as dielectric constant, viscosity, auto-protolysis constant, polarity and volatility can be successfully exploited to adjust the selectivity upon modifying the electrophoretic mobilities. Thus, NACE not only allows the separation of analytes that are insoluble in water, but also might enable the separation of structurally very closely related compounds [8,9]. However, a limited number of publications are dedicated to the separation of alkaloids by NACE. For example, Cherkaoui et al. described the application of NACE for the analysis of tropane alkaloids [10], Barthe et al. studied the separation of *Vinca* indol-alkaloids [11], Li et al. of representatives of the quinolizidine group in Chinese herbs [12] and later also of three aconitine alkaloids [13]. Gao et al. published most recently two NACE-methods for the analysis of the protoberberines berberine, palmatine and jatrorrhizine [14] as well as for two bisbenzylisoquinoline alkaloids, namely fangchinoline and tetrandrine [15].

Hyphenation of capillary electrophoresis with a mass spectrometer using an electrospray ionization interface (CE–ESI-MS) represents a powerful combination of high separation efficiency with high detection sensitivity. Additionally mass spectrometry has a great capability for molecular

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characterization [16–18]. Particularly, NACE is well compatible with ESI-MS in terms of enhanced sample ionization process resulting in improved sensibility compared to separations in aqueous buffer systems. However, only very few studies have demonstrated the potential of NACE–MS for the separation and determination of alkaloids up to now. Two publications are dedicated to steroidal glycoalkaloids as solanidin and related compounds in *Solanum* species [19,20], Liu et al. separated alkaloids from *Stephania tetrandra* [21] and Unger et al. applied NACE–MS for a preliminary characterization of naphthylisoquinoline alkaloids in *Ancistrocacladus* species [22].

The aim of this study was the development of an analytical method which does not only allow the separation and characterization of the various structural subtypes of isoquinoline alkaloids in *F. herba* extracts and phytopharmaceuticals, but also the standardization of these compounds. This paper demonstrates that non-aqueous capillary electrophoresis coupled to an ion trap mass spectrometer is capable to meet all these demands.

2. Experimental

2.1. Materials

Acetic acid, ammonium acetate, ammonium formate, diethyl ether, formic acid, sodium hydroxide (all analytical reagent-grade), acetonitrile, isopropanol and methanol (all HPLC gradient grade) were purchased from Merck (Darmstadt, Germany). Water was produced by reverse osmosis followed by distillation. Nitrogen (99.995%) for mass spectrometry was produced by a nitrogen generator (Peak Scientific Instruments, Fountain Crescent, UK). Reference compounds **1–10** (see Fig. 1) were isolated from *F. officinalis* and identified by IR, UV, NMR and MS [23]. Compounds **a** (presumably fumaritine) and **b** (presumably fumaricine) were isolated but could not be unequivocally identified. *F. officinalis* (*F. herba*) was purchased at Leopold Bichler KG (Innsbruck, Austria). Two commercially available phytotherapeutics containing extracts of *F. officinalis* were purchased in a local pharmacy. The weight of the extract was indicated as 250 mg/tablet for both preparations. Voucher specimens are deposited at the Institute of Pharmacy, University of Innsbruck, Austria.

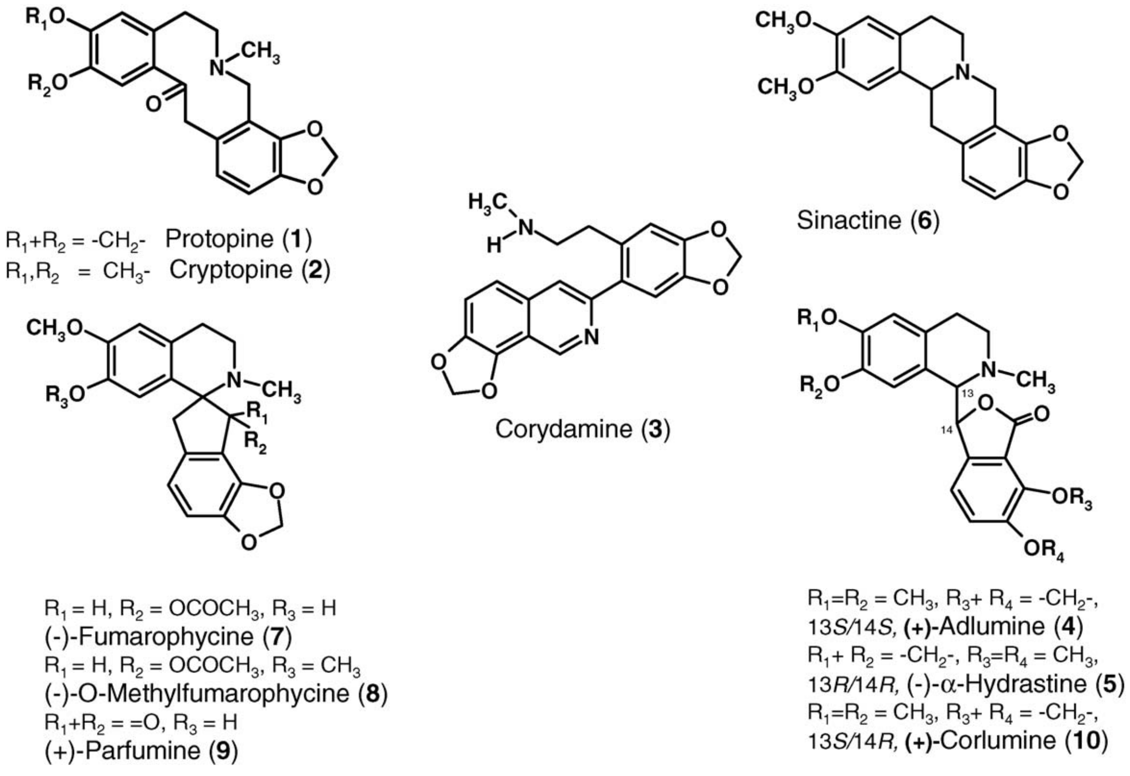
2.2. Sample preparation

2.2.1. Extract A (for optimization of CE–MS parameters and isolation of reference compounds)

Four hundred and fifty grams of powdered plant material was extracted with methanol using a Soxhlet apparatus. The solvent was evaporated at reduced pressure and the residue was dissolved in 0.02 M HCl. After liquid–liquid partition with diethyl ether, alkaloids were extracted from the basified aqueous phase (pH 10 with 0.1 N NaOH) with diethyl ether (1.85 g). For the optimization experiments 1 mg of the alkaloid fraction was dissolved in 1 ml methanol.

2.2.2. Extract B (for quantitative determinations)

5.608 g powdered plant material was extracted exhaustively with methanol under reduced pressure using a small Soxhlet



R$_1$+R$_2$ = -CH$_2$- Protopine (**1**)
R$_1$,R$_2$ = CH$_3$- Cryptopine (**2**)

Corydamine (**3**)

Sinactine (**6**)

R$_1$ = H, R$_2$ = OCOCH$_3$, R$_3$ = H
(-)-Fumarophycine (**7**)
R$_1$ = H, R$_2$ = OCOCH$_3$, R$_3$ = CH$_3$
(-)-O-Methylfumarophycine (**8**)
R$_1$+R$_2$ = =O, R$_3$ = H
(+)-Parfumine (**9**)

R$_1$=R$_2$ = CH$_3$, R$_3$+ R$_4$ = -CH$_2$-,
13*S*/14*S*, **(+)**-Adlumine (**4**)
R$_1$+ R$_2$ = -CH$_2$-, R$_3$=R$_4$ = CH$_3$,
13*R*/14*R*, (-)-α-Hydrastine (**5**)
R$_1$=R$_2$ = CH$_3$, R$_3$+ R$_4$ = -CH$_2$-,
13*S*/14*R*, **(+)**-Corlumine (**10**)

Fig. 1. Structures of *Fumaria officinalis* isoquinoline alkaloid derivatives **1–8**.

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