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Qualitative and quantitative analysis of quaternary ammonium alkaloids from Rhizoma Corydalis by matrix-assisted laser desorption/ionization Fourier transform mass spectrometry coupled with a selective precipitation reaction using Reinecke salt

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Abstract

A major problem hampering the use of MALDI-MS for quantitative measurements is the inhomogeneous distribution of analytes and matrices in sample preparations. In this study, an aerospray method was utilized for sample preparation method to improve sample homogeneity across stainless steel targets for quantitative analysis of quaternary ammonium alkaloids (QAAs). A selective precipitation reaction with Reinecke salt known to selectively trap QAAs was used to facilitate the separation and purification of QAAs from the complex crude plant extracts. Palmatine and berberine as the representative QAAs in commercial Rhizoma Corydalis were successfully quantified by introducing an internal standard with similar molecular properties as analytes. The LODs were found to be 0.07 fmol, for palmatine, and 0.24 fmol, respectively, for berberine. The content of QAAs of three commercial Rhizoma Corydalis was between 0.201 and 0.245% for palmatine, and 0.049–0.057% for berberine. Furthermore, MS/MS experiments based on the accurate-mass measurements were carried out by infrared multiphoton dissociation (IRMPD) for QAAs and the corresponding tertiary alkaloids, which offered additional selectivity for this quantitative analysis method. In the fragmentation of precursor ions from QAAs, only cleavage of substituted groups attached to the A- or D-ring was observed, while cleavage between B- and C-ring from tertiary alkaloids had occurred. This study offers a perspective into the utility of MALDI-FTMS as an alternate quantitative tool for QAAs, especially in complex plant extracts.

Keywords: MALDI-MS; *Corydalis yanhusuo*; Quantitative analysis; Reinecke salt; Aerospray

1. Introduction

Quaternary ammonium alkaloids (QAAs) of many medicinal plants are important plant metabolites with significant effects on inhibition of aminopeptidase N and dipeptidyl peptidase IV [1], and on neuromuscular transmission [2], as well as exhibiting antibacterial, antiamoebic, antifungal, and antihelminthic properties [3], and cytotoxicity in vitro against tumor cells [4,5]. Due to their high polarity, relatively high basicity and poor volatility, a direct analysis of QAAs by GC or routine HPLC is not easy and it is not possible to easily control their content. Recently, electrophoretic separation [6–9] and liquid chromatographic separation on ion-pair mechanism or other special columns [10,11] have been reported, with partly promising results for determination of QAAs.

Rhizoma Corydalis (*Corydalis yanhusuo* W.T. Wang) is employed in traditional Chinese medicines as an analgesic agent for treating spastic pain, abdominal pain, menstrual pain, and pain due to injuries [12]. Previous phytochemical studies have shown that protoberberine-type alkaloids (QAAs) and tetrahydroprotoberberine-type (tertiary) alkaloids have been identified as the active secondary metabolites of the plant (Fig. 1) [13,14]. Previously, much attention had been paid to analysis of the tertiary alkaloids by routine HPLC, and to some modified

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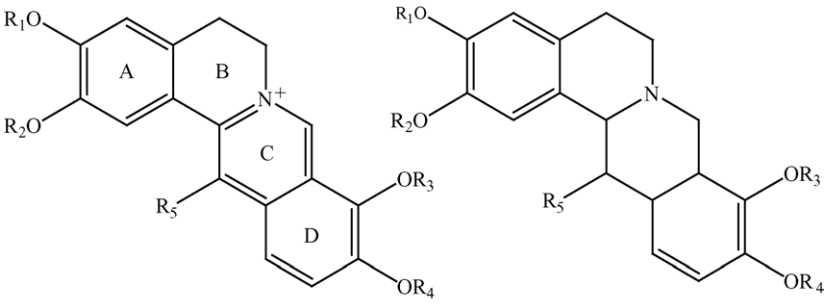

1. $R_1+R_2=R_3+R_4=CH_2$ $R_5=H$
2. $R_1=R_2=R_3=R_4=R_5=CH_3$
3. $R_1=R_2=R_3=R_4=CH_3$ $R_5=H$
4. $R_1=R_3=R_4=CH_3$ $R_2=R_5=H$
5. $R_1+R_2=CH_2$ $R_3+R_4=CH_3$ $R_5=H$
6. $R_1=R_2=R_3=R_4=R_5=CH_3$
7. $R_1=R_2=R_3=R_4=CH_3$ $R_5=H$
8. $R_1+R_2=CH_2$ $R_3+R_4=CH_3$ $R_5=H$

Fig. 1. Some known QAAs (1–5) and tertiary alkaloids (6–8) in Rhizoma Corydalis.

HPLC methods for several QAAs in the plant [15,16], however, which no fully satisfactory methods had been found to give a complete analysis of the total QAAs. Recently, some novel methods based on electrospray ionization mass spectrometry (ESI-MS) and electrophoretic principles have been successfully developed [17–19].

Recently, quantitative MALDI mass spectrometry (MALDI-MS) has been widely developed in most fields of life science for macromolecules; however the field of quantitative analysis of small molecules is still emerging, especially for compounds with molecular weights in the matrix region ($m/z < 500$) [20–23]. Also, MALDI-MS has a major disadvantage with respect to its low reproducibility in quantitative analysis. Many researchers had made improvements in the sample deposition technology (over the conventional dried-droplet method), including use of an electrospray device [24], introduction of an internal standard [25], electrospray deposition on porous silicon [26], fast evaporation technology [27], vacuum crystallization [28], and aerospray method [29]. For example, Wilkins and co-workers [29–31] have reported an aerospray method by using a home-built spray device, which gas flowed through a stainless steel tube and coaxially over a fused-silica capillary through which the sample was infused. In the present work, a modified aerospray setup was developed, which an elecrospray tube supplied by an Agilent 1100 LC/MSD mass spectrometer served as the coaxial tube and the capillary. This aerospray method afforded very homogeneous analyte spots, and at the same time, made quantitative MALDI method easy in any mass spectrometry laboratory. In the present work, this method of sample preparation for MALDI was coupled with an internal standard and a selective chemical precipitation reaction for QAAs in order to analyze these compounds directly in extracts of Rhizoma Corydalis by MALDI-MS. In addition, IRMPD-MS/MS experiments were performed to elucidate the fragmentation pathways of QAAs and the corresponding tertiary alkaloids; obvious differences were observed, and the two types of alkaloids could thereby be easily distinguished. In addition these experiments permitted partial structural elucidation that enhanced the selectivity of this quantitative method.

2. Experimental

2.1. Reagents and standard alkaloids

Optima-grade acetone and acetic acid were obtained from Shanghai Reagent Company, China. The deionized water used in this study was from a Milli-Q water purification system (Millipore Corp., Bedford, MA, USA). Two QAA standards (palmatine chloride, PC; berberine chloride, BC), and a standard tertiary alkaloid (tetrahydropalmatine, TP), were purchased from the National Institute for the Control of Pharmaceutical and Biological Products, China. Tetraethyl ammonium bromide (the internal standard), 2,5-dihydroxybenzoic acid (DHB) and PEG-400 were purchased from Sigma. Reinecke salt ($NH_4[Cr(NH_3)_2(CNS)_4]H_2O$) was purchased from Shanghai No. 2 Reagent Company, China. A saturated solution of Reinecke salt was freshly prepared by dissolving 4 g of Reinecke salt in 60 mL deionized water; this was filtered before use.

2.2. Plant materials

The Rhizoma Corydalis were purchased from Shanghai Lei-Yun-Shang Chinese Medicine Drinking Pills Factory, China, and identified by Prof. Wang Zheng-tao, the Department of Traditional Chinese Medicines, China Pharmaceutical University. The voucher specimen (YHS-2003-12) has been deposited at the Herbarium of the Department of Traditional Chinese Medicines, China Pharmaceutical University.

2.3. Sample preparation

A volume of 5 mL 1% acetic acid was used to extract QAAs from the dried powders of Rhizoma Corydalis (500 mg) in a