Journal of Chromatography B 1096 (2018) 73–79

Contents lists available at ScienceDirect

Journal of Chromatography B

journal homepage: www.elsevier.com/locate/jchromb

Analysis of alkaloids from *Peganum harmala* L. sequential extracts by liquid chromatography coupled to ion mobility spectrometry

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ARTICLE INFO

Keywords:
HPLC
Ion mobility spectrometry
Tandem mass spectrometry
HILIC column
Peganum harmala L.
Alkaloids

ABSTRACT

An orthogonal two dimensional analysis method based on high performance liquid chromatography (HPLC) separation and electrospray ionization-ion mobility spectrometry (ESI-IMS) detection was developed for the analysis of alkaloid compounds from *Peganum harmala* L. seeds. Reverse phase (RP) and hydrophilic interaction chromatography (HILIC) were compared for the most optimal performance using three different chromatographic columns. The experimental results suggest that HILIC mode is a better option for combining with the ESI-IMS system for higher sensitivity and ease in hyphenating. Under optimized conditions, alkaloids from different extraction phases were determined by means of the established HPLC-IMS method. More compounds from *Peganum harmala* L. seed extracts were differentiated on the HPLC-ESI-IMS system by their retention time and drift time than by HPLC or ESI-IMS alone, and thirteen alkaloids were tentatively identified based on m/z and fragment ions using ultra-high-performance liquid chromatography tandem mass-spectrometry (UPLC-MS/MS). Hence, our results indicate that this method can be considered to be advantageous over traditional absorbance detection methods for resolving complex mixtures because of complementary separation steps, elevated peak capacity, and higher sensitivity.

1. Introduction

Alkaloids are a type of alkaline organic compound found in nature, mainly in plants, animals, fungi, bacteria, and other organisms [1]. Most of them have complex ring structures that contain nitrogen as a heteroatom in the molecule [2]. So far, more than 10,000 alkaloids have been isolated from the natural kingdom, mainly found in higher plants, especially in the dicotyledonous plants [3], but there are also some alkaloids in the lower plants [1,4]. Alkaloids play a vital role in living organisms and are considered to offer protection for plants [5]. Nevertheless, the most attractive and outstanding feature of alkaloids may be attributed to their significant biological and pharmacological activities. In traditional Chinese medicine, alkaloids are often one of the most important ingredients due to their unique activities [1].

Peganum harmala L., which is called harmal, esfand, aspand, wild rue, Syrian rue, or African rue, is an important member of the family of Zygophyllaceae, and is a perennial herbaceous, glabrous plant that spontaneously grows in arid and semiarid regions, steppe areas, and sandy soils [6,7]. This herbaceous plant is not only native to the eastern Mediterranean region but also widely distributed in Asia, North Africa, and America [8–10]. In China, this plant has long been used as a traditional folk medicine to treat various ailments, including cough, asthma, rheumatism, hypertension, diabetes, jaundice, apoplexia, lumbago, arthralgia, amnesia, hemiplegia, enteritis, dysentery, malaria, and some skin diseases [7,10,11]. It is also a traditional medicine that is used as a central nervous system (CNS)-stimulating agent [12], abortifacient agent, emmenagogue [13], and anti-inflammatory agent [14] in Turkey, North Africa, Middle East, and Spain, respectively.

Previous reports have indicated that the pharmacologically active compounds in *P. harmala* are β-carboline alkaloids and quinazoline alkaloids, which are primarily found in the seeds and roots [15,16]. Up to now, those compounds have exhibited various bioactivities, such as antimicrobial, antimalarial, antitumoral, anticancer, and antioxidant activities, anti-inflammatory, analgesic, cytotoxic, and immunomodulator properties, and enzyme inhibition, cardiovascular actions, and some other activities [7,8,16,17].

Various approaches, often employing chromatographic separation and analysis, have been used for the determination of β-carboline

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https://doi.org/10.1016/j.jchromb.2018.08.021
Received 10 April 2018; Received in revised form 19 August 2018; Accepted 20 August 2018
Available online 21 August 2018
1570-0232/

Table 1
Gradient elution conditions and instrument parameters of different chromatographic columns.

Column	X-Bridge HILIC column			X-Bridge RP C_{18} column			PCP RP C_{18} column		
	Times (min)	ACN (%)	0.6% NH_4OH (%)	Times (min)	ACN (%)	0.6% NH_4OH (%)	Times (min)	ACN (%)	0.1% HCOOH (%)
Chromatographic eluting gradient conditions	0	95	5	0	5	95	0	5	95
	30	65	35	10	55	45	10	30	70
	30.01	Stop		30	55	45	25	30	70
				30.01	100	0	35	100	0
				45	Stop		45	Stop	
Flow rate	0.3 mL/min			1 mL/min			1 mL/min		
Injection volume	2 μL			20 μL			20 μL		
Wavelength	220 nm			220 nm			254 nm		
Column temperature	35 °C			35 °C			35 °C		

alkaloids and quinazoline alkaloids. For instance, thin-layer chromatography (TLC), capillary electrophoresis (CE), and high performance liquid chromatography (HPLC) methods in combination with electrochemical, chemiluminescence, ultraviolet (UV), photodiode array (DAD), or fluorescence as well as gas chromatography (GC), gas chromatography–mass spectrometry (GC–MS), high performance liquid chromatography coupled to mass spectrometry (HPLC-MS), capillary electrophoresis-mass spectrometry (CE-MS), two-dimensional liquid chromatography (2D LC), and other techniques [8,10,18–22].

One disadvantage of liquid chromatography is that when the chemical content is very complex, it becomes increasingly difficult to achieve sufficient separation due to limited resolution and peak capacity [22,23]. Recently, LC-MS has been increasingly used in the analysis of complex biological and pharmaceutical samples, but this combination mode still presents a series of challenges, such as an insufficient ability to separate isomers with identical mass-to-charge ratio. Similarly, comprehensive 2D LC is still deficient because of the incompatibility of different separation modes, time-consuming gradient elution, specific interfaces, and difficult operation [24].

Ion mobility spectrometry (IMS) is a post-ionization separation technique based on the shape, size, and collision cross-section of ions. Combined with electrospray ionization, IMS has been successfully applied to the analysis of volatile compounds. To the best of our knowledge, there is no hyphenated HPLC-IMS method for the analyses of β-carboline alkaloids and quinazoline alkaloids. Therefore, the aim of this work is to develop a novel approach based on high performance liquid chromatography (HPLC) and ion mobility spectrometry (IMS) for the analysis of alkaloids from the seeds of *P. harmala*. This orthogonal analysis method provides an increased peak capacity without an increase in the analysis time compared to 2D LC [25,26].

2. Materials and methods

2.1. Material, standards, and reagents

The seeds of *P. harmala* were purchased from Ankang Siji Health Protection Yangsheng Tang (Anhui, China) and were authenticated by associate professor WenJuan Huang, College of Life Science of Tarim University, China. LC-MS grade acetonitrile (ACN) and methanol (MeOH) were obtained from Fisher Scientific (Ottawa, Canada) and filtered with a 0.22-μm organic membrane before using. Analytical grade ethanol, chloroform, ethyl acetate, n-butanol, formic acid (HCOOH), hydrochloric acid (HCl), and ammonium hydroxide (NH_4OH) were provided by Tianjin Zhiyuan Reagent Co., Ltd. (Tianjin, China). Water was processed with a Milli-Q water purification system (Shanghai, China).

2.2. Liquid chromatography

The Shimadzu LCAT-20 HPLC consists of a dual solvent pump high-pressure gradient system, SIL-20AT auto sampler, CBM-20A integrator, SPD-M20A UV–Vis diode array detector, and CTO-20A column oven (Kyoto, Japan). Samples were separated on a 2.1 mm * 100 mm, 5 μm particle, Waters X-Bridge HILIC column, a 4.6 mm * 250 mm, 5 μm particle, Waters X-Bridge $C1_8$ column (Dublin, Ireland) with a guard column, and a 4.6 mm*150 mm, 5 μm particle, Acchrom PCP C_{18} column (Wenling, China), respectively. A T-connector was used as a post-column splitter, and the specific gradient conditions are summarized in Table 1. The data analysis was performed using Labsolutions LC software.

2.3. Ion mobility spectrometry

In this study, a drift tube electrospray ionization ion mobility spectrometer was employed, and it has been previously described in detail elsewhere [27,28]. The IMS instrument is composed of an IMS tube, two Bertan 205B high voltage power supply boxes obtained from Bertan (Hicksville, USA), a Keithley 427 amplifier (Cleveland, USA), and in-house-made Labview (Austin, USA)-based data acquisition (DAQ) software. The IMS tube consisted of a 6.4 cm de-solvation region and a 16.8 cm drift region that are separated by a Bradbury–Nielsen type ion gate. The voltages applied to the electrospray ionization (ESI) needle and drift tube were set at 4.5 kV and 7.5 kV, respectively. The IMS tube was maintained at a constant temperature of 95 °C and operated at atmospheric pressure, which was 728 Torr for Alar, Xinjiang. Clean air was used as drift gas, held at a constant flow rate of approximately 1.5 L/min. A 150-μm inner diameter silica capillary tubing with polyimide coating was used to conduct the eluent from the HPLC column to a T-piece, and 6-cm-long 20-μm inner diameter silica capillary tubing was used as an ESI needle. The specific HPLC-IMS online combined device diagram is shown in Fig. 1.

2.4. UPLC-MS/MS analysis

The peaks of alkaloids, which were generated from the HPLC-ESI-IMS analysis, were further identified by the HPLC-ESI-MS/MS system and compared with published mass data. LC separation was performed on a Waters Acquity UPLC TQD triple quadrupole mass spectrometer with the same LC X-Bridge HILIC column parameters as those described in Table 1. The ESI source was used in positive ion mode, and the automatic parameters were set as follows: the cone gas was 1 L/h^{-1}, the desolvation temperature at 500 °C, the desolvation gas flow was 1000 L/h-1, and the source temperature was 150 °C. The precursor ions were determined using a full scan mode with *m/z* from 50 to 750, the cone voltage set at 70 V for the precursor ion scan, and collision voltage set at 30 V for the product ion scan. Masslynx software (Waters) was used for data acquisition and instrument control.