

# 超声萃取雾化碳纤维离子化质谱快速区分 当归和欧当归

吕海山<sup>1,2</sup>, 张明童<sup>3</sup>, 苏越<sup>1</sup>, 郭寅龙<sup>2</sup>

(1. 上海中医药大学交叉科学研究院, 上海 201203;

2. 中国科学院上海有机化学研究所, 上海有机质谱中心, 上海 200032;

3. 甘肃省药品检验研究院, 甘肃 兰州 730030)

**摘要:** 由于当归与欧当归外形相似, 常被不法商贩混淆使用。本研究采用超声萃取雾化碳纤维离子化质谱 (UENRT/CFI-MS) 法研究当归和欧当归的成分差异, 以快速区分二者。以甲醇作为提取溶剂, 将药材切片样品置于超声雾化片上, 利用超声雾化片的高频振荡实现对样品的在线萃取、过滤和雾化。雾化后的中药样品小液滴在施加高压的碳纤维附近被电离, 随后对 2 种药材的质谱图进行比较分析, 寻找差异成分。结果表明, 在欧当归的质谱图中识别到  $m/z$  496 特征峰, 而在当归中未检测到该峰, 这一结果为区分 2 种药材提供了依据。该方法无需繁琐复杂的样品前处理过程, 具有分析速度快、操作简单、绿色环保、灵敏度高等优点, 能够有效快速区分当归及其伪品欧当归。

**关键词:** 当归; 欧当归; 质谱; 直接分析; 敞开式离子化; 超声萃取雾化; 碳纤维离子化

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## Rapid Differentiation of *Angelica sinensis* and *Levisticum officinale* by Ultrasonic Extraction and Nebulization in Real-Time/Carbon Fiber Ionization Mass Spectrometry

LYU Hai-shan<sup>1,2</sup>, ZHANG Ming-tong<sup>3</sup>, SU Yue<sup>1</sup>, GUO Yin-long<sup>2</sup>

(1. Institute for Interdisciplinary Medicine Sciences,

Shanghai University of Traditional Chinese Medicine, Shanghai 201203, China;

2. National Center for Organic Mass Spectrometry in Shanghai, Shanghai Institute of Organic Chemistry,

Chinese Academy of Sciences, Shanghai 200032, China;

3. Gansu Institute of Drug Control, Lanzhou 730030, China)

**Abstract:** The rapid and accurate differentiation of *Angelica sinensis* and *Levisticum officinale* is a significant challenge in the quality control of traditional Chinese medicine. *Angelica sinensis* and *Levisticum officinale* are different genera of the same family. Due to their similar appearances and partially shared chemical components, unscrupulous traders often sell counterfeit *Levisticum*

*officinale* as *Angelica sinensis* to make exorbitant profits. In this study, a fast and easy method of ultrasonic extraction and nebulization in real-time/carbon fiber ionization mass spectrometry (UENRT/CFI-MS) was introduced for the swift discrimination of these two easily confused traditional Chinese medicines. UENRT/CFI-MS primarily consists of an online extraction and nebulization unit, an ionization unit. The online extraction and nebulization unit employ an ultrasonic nebulizer chip for real-time sample extraction and nebulization. The ionization unit comprises a 10 mm long carbon fiber rod nested in a metal tube, achieving ionization through the application of a 3 kV high voltage, and the auxiliary solvent (methanol) is continuously supplied to the carbon fiber at a rate of 5  $\mu\text{L}/\text{min}$ . Slices of *Angelica sinensis* and *Levisticum officinale* from different origins with similar size, appearance, and quality (diameter less than 16 mm, thickness not exceeding 0.5 cm) were selected and placed directly on the nebulizer chip. For each analysis, 200  $\mu\text{L}$  methanol was continuously added using a pipette. When the nebulizer was activated, the lower end of the nebulizer chip generated a continuous spray of tiny droplets towards the carbon fiber tip for several seconds, which were then ionized before entering the mass spectrometer for detection. This device integrates online ultrasonic extraction, nebulization, and carbon fiber ionization technologies, enabling real-time analysis of complex samples without cumbersome pretreatment. By applying this technique, a characteristic ion peak of  $m/z$  496 is uniquely identified in *Levisticum officinale*, serving as a distinctive marker for differentiation of *Angelica sinensis* and *Levisticum officinale*. Subsequently, comparative analysis was conducted using sample extracts on electrospray ionization mass spectrometry (ESI-MS) and carbon fiber ionization mass spectrometry (CFI-MS). These experimental results showed that CFI-MS exhibits superior selectivity and efficiency in detecting the  $m/z$  496 characteristic peak, which is not observable in ESI-MS analysis. This finding highlights the unique ability of carbon fiber ionization to selectively detect certain compounds. The UENRT/CFI-MS technique was applied for the direct analysis of *Angelica sinensis* and *Levisticum officinale*, and can obtain the analysis results in just a few seconds. A distinctive ion of  $m/z$  496 is detected in *Levisticum officinale*, facilitating swift discrimination between *Angelica sinensis* and *Levisticum officinale*. However, further research is needed to elucidate the structure of the  $m/z$  496 characteristic peak. Additionally, the method's applicability to a wider range of herbal medicines and its performance in more complex matrices should be investigated. This method is expected to play a role in traditional Chinese medicine identification.

**Key words:** *Angelica sinensis*; *Levisticum officinale*; mass spectrometry; direct analysis; ambient ionization; ultrasonic extraction and nebulization in real-time; carbon fiber ionization mass spectrometry

当归 (*Angelica sinensis* (Oliv.) Diels) 是伞形科植物的干燥根<sup>[1]</sup>, 味甘、辛, 性质温和, 具有补血活血、调经止痛、润肠通便之功效, 是中医药领域的重要药材之一。在传统中医理论中, 当归被赋予“十方九归”的美誉, 凸显了其在临床应用中的重要地位。与之相对, 欧当归 (*Levisticum officinale*) 虽属同科, 但归于不同属, 其性质稍温, 以辛味为主, 主要用于活血调经和利尿。尽

管两者在植物分类上有一定的相似性, 但其药性和应用却存在显著差异。值得注意的是, 因欧当归辛燥的特性, 使用时常引发一些不良反应, 如恶心、头晕或血热失衡等。这些副作用与当归温和滋养的特性形成鲜明对比, 两者在应用中不可互换。然而, 由于欧当归栽培相对简单, 生长周期较短 (通常为 180~200 天), 一些不法商贩将其冒充为正品当归出售<sup>[2-3]</sup>, 这不仅影响治疗

效果,还有可能危及患者的生命安全。因此,开发一种快速、准确区分当归和欧当归的方法至关重要。

中药材饮片的真伪鉴定一直是传统医药领域的重要课题。长期以来,主要依赖性状观察、显微检查和理化分析等方法进行鉴别<sup>[4-5]</sup>。然而,这些传统技术的有效性受限于检验人员的专业素养和实践经验。在当归中掺伪欧当归的鉴别中,由于两者形态相近,仅凭传统方法难以准确区分,尤其在经过饮片加工后,鉴别难度显著增大。近年来,针对当归和欧当归的检测方法有了进一步发展,史中飞等<sup>[6-7]</sup>建立了特异性聚合酶链式反应(PCR)和聚合酶链式反应-限制性片段长度多态性(PCR-RFLP)鉴别当归药材及饮片中掺伪欧当归。车苏容等<sup>[8]</sup>应用紫外吸收光谱对当归和欧当归进行鉴别,结果表明,二者的石油醚、氯仿提取液无明显差异,仅在水乙醇和蒸馏水提取液中存在差异。这些方法虽然在一定程度上提高了鉴别准确性,但仍存在需要繁琐的样品前处理过程,无法实现实时原位检测,需要使用大量有机溶剂等局限性。

中药具有体系复杂、化学成分多样等特性,这不仅与中药材的来源和加工过程有关,还包括其活性成分在不同生长环境和季节中的变化,因此,分析其特定成分面临诸多挑战。质谱凭借高灵敏度、高特异性和高分辨率的特性,能够有效识别和定量中药中的多种化合物<sup>[9-12]</sup>,同时,通过与其他分离技术联用,可以更全面地表征中药成分<sup>[13-16]</sup>。刘淑莹课题组<sup>[17]</sup>应用直接实时分析-四极杆-轨道阱质谱技术快速鉴别五味子和南五味子,通过优化实验条件和使用偏最小二乘判别分析,不仅实现了2种样品的有效区分,还识别出关键的差异化合物。张强等<sup>[18]</sup>利用碳纤维电离质谱(CFI-MS)直接分析未经前处理的中药挥发性成分,成功表征了多种中药及复方制剂中的多类化合物,并快速鉴别中药材的新鲜程度。Wang等<sup>[19]</sup>使用高效液相色谱-质谱和气相色谱-质谱结合多变量统计分析,对南柴胡和北柴胡中5种皂苷进行定量分析,为不同品种柴胡的鉴别以及质量评价提供了参考。吴学峰等<sup>[20]</sup>利用超高效液相色谱-四极杆-串联飞行时间质谱法对蒸制佛手的化学成分进行分析,为该药材的标准建立和药效研究提供了数据支持。

本课题组开发了超声雾化碳纤维离子化质谱(UENRT/CFI-MS)技术,通过在线超声萃取与碳纤维离子化相结合,实现了样品中内部成分的实时萃取、雾化和检测,并成功应用于中药中非法添加合成药的快速筛查<sup>[21]</sup>。本研究将采用UENRT/CFI-MS技术区分当归和欧当归,利用超声萃取雾化装置的高频振荡实现对中药和中药制品的在线萃取、在线雾化,被雾化后的中药样品分子到达施加高压的碳纤维离子源附近被电离,随后进入质谱口完成检测,旨在当归和欧当归的快速鉴别提供新的解决方案。

## 1 实验部分

### 1.1 主要仪器与装置

TSQ Quantum Access 三重四极杆质谱仪:美国赛默飞世尔科技公司产品;超声波清洗器:昆山超声波仪器有限公司产品;超声雾化片(16 mm):深圳市象宇科技电子有限公司产品;高压电源器:宿迁市波尔高压电源有限公司产品;针头式过滤器(0.22 μm):上海安谱科技有限公司产品;USB充电头:东莞市奥海科技股份有限公司产品;碳纤维:日本TORAY Industries公司产品;Milli-Q型纯水仪:美国Millipore公司产品;5810 R离心机:德国Eppendorf公司产品。

本实验室开发的UENRT/CFI-MS装置示意图和整体结构示于图1。UENRT/CFI离子源主要由在线提取雾化单元和离子化单元组成。其中,在线提取雾化单元采用超声雾化片,用于样品的实时提取和雾化;离子化单元由1根嵌套在金属管的长10 mm碳纤维棒构成,通过施加高压实现离子化过程。

在操作过程中,辅助溶剂甲醇以5 μL/min持续输送到碳纤维上。碳纤维被精确定位在质谱入口的正前方,并与入口保持水平。超声雾化片则位于碳纤维尖端和质谱入口之间的上方位置。

具体参数设置如下:碳纤维施加电压3.0 kV;超声雾化片与质谱入口的垂直距离为6 cm;碳纤维尖端与质谱入口的水平距离为3 mm;离子传输管温度为275 ℃。

### 1.2 主要材料与试剂

甲醇:色谱级,德国Merck公司产品;当归、欧当归:甘肃省药品检验研究院产品,经甘肃省

药品检验研究院鉴定分别为伞形科植物当归 *Angelica sinensis* (Oliv.) Diels. 的干燥根和伞形科

植物欧当归 *Levisticum officinale* 的干燥根茎。样本信息列于表1。

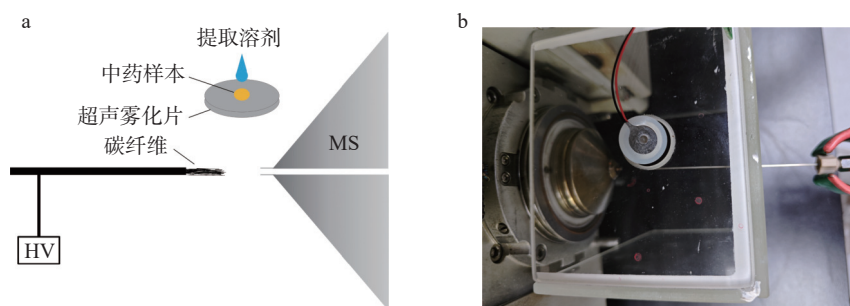


图1 超声萃取雾化碳纤维离子源示意图(a)和实物图(b)

Fig. 1 Diagram (a) and actual image (b) of UENRT/CFI-MS

表1 当归和欧当归的信息

Table 1 Information of *Angelica sinensis* and *Levisticum officinale*

中药材 Traditional Chinese medicinal material	产地 Place of origin	批次 Batch
当归	民乐、云南、漳县、岷县、马峡镇寺沟村、马峡镇车厂沟村、 马峡镇蒋庄村、马峡镇孟台村、马峡镇腰崖村	6
欧当归	甘肃渭源县	6

### 1.3 实验条件

质量扫描范围  $m/z$  10~1 000, 离子传输管温度 275 °C, 离子源真空  $5.72 \times 10^{-4}$  Pa。

### 1.4 实验方法

选择大小、外观、质量相近的不同产地当归和欧当归饮片(直径小于 16 mm, 厚度不超过 0.5 cm), 直接置于雾化元件上。添加 200  $\mu$ L 甲醇作为提取剂, 启动雾化装置进行在线提取。样品被雾化成微小液滴, 喷向带电碳纤维尖端实现离子化, 随后进入质谱仪分析。

选取不同产地当归和欧当归饮片, 以甲醇作为提取溶剂。采用常规溶剂提取法, 先经超声波清洗机超声提取 30 min, 随后在 4 °C 下以 12 000 r/min 离心 10 min, 取上清液, 过 0.22  $\mu$ m 针头过滤器, 得到提取液。将提取液分为 2 组, 第 1 组采用 CFI-MS 分析, 第 2 组采用 ESI-MS 分析。

## 2 结果与讨论

### 2.1 当归和欧当归的 UENRT/CFI-MS 质谱图比较

目前, 对当归和欧当归的比较研究主要聚焦于形态和显微结构特征<sup>[22-23]</sup>。在化学成分方面, 对欧当归的全面系统研究不足。现有文献表明<sup>[24-25]</sup>, 当归和欧当归在某些化学成分上存在相

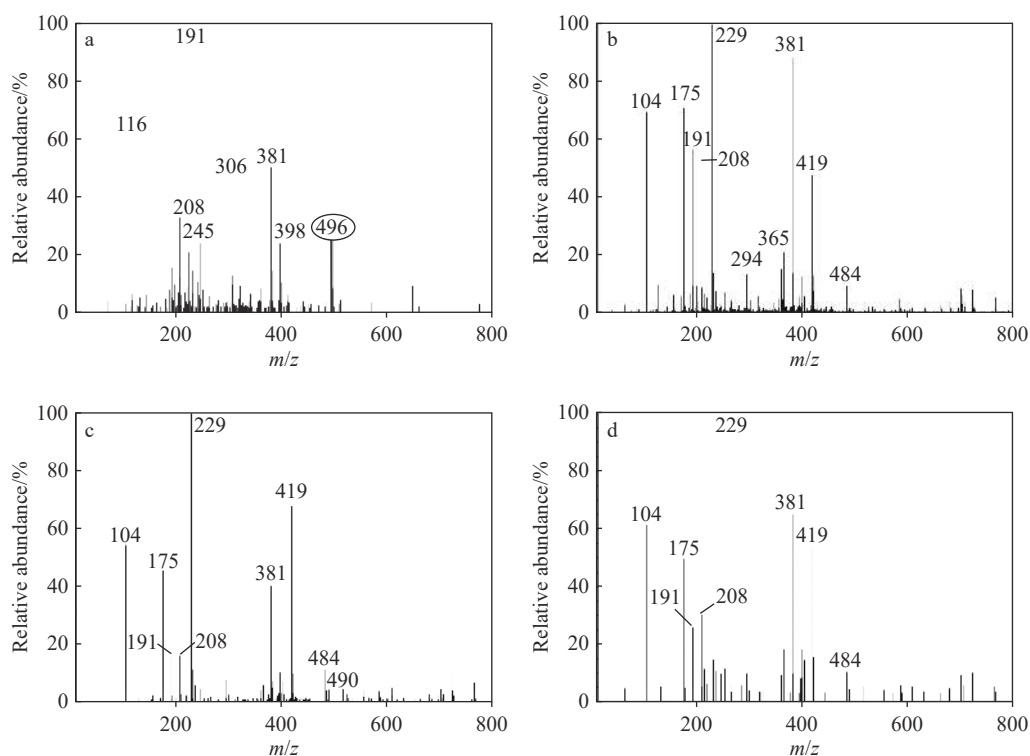
似性, 如, 均含有机酸、丁苯酚类化合物和氨基酸等。但是, 二者的具体成分差异尚未得到充分阐明。

当归和欧当归的 UENRT/CFI-MS 质谱图示于图 2, 均能观察到蒿本内酯( $m/z$  191)、新当归内酯( $m/z$  381)等活性成分的特征峰。此外, 发现欧当归中显示出  $m/z$  496 特征峰, 而在当归中没有检测到。为避免误差, 对样本进行多次重复实验, 确定  $m/z$  496 特征峰仅出现于欧当归的质谱图。

### 2.2 UENRT/CFI-MS 与 ESI-MS 的对比

为深入探究 UENRT/CFI-MS 法在当归和欧当归鉴别中的优势, 本研究首先通过样品的甲醇提取液进行 ESI 检测, 当归和欧当归的图谱相似, 难以区分, 且在欧当归的图谱中也没有检测出  $m/z$  496 离子峰, 示于图 3a、3b。CFI-MS 能够检测到欧当归中  $m/z$  496 特征峰, 这一差异表明了 CFI-MS 在特定成分分析中的独特优势。

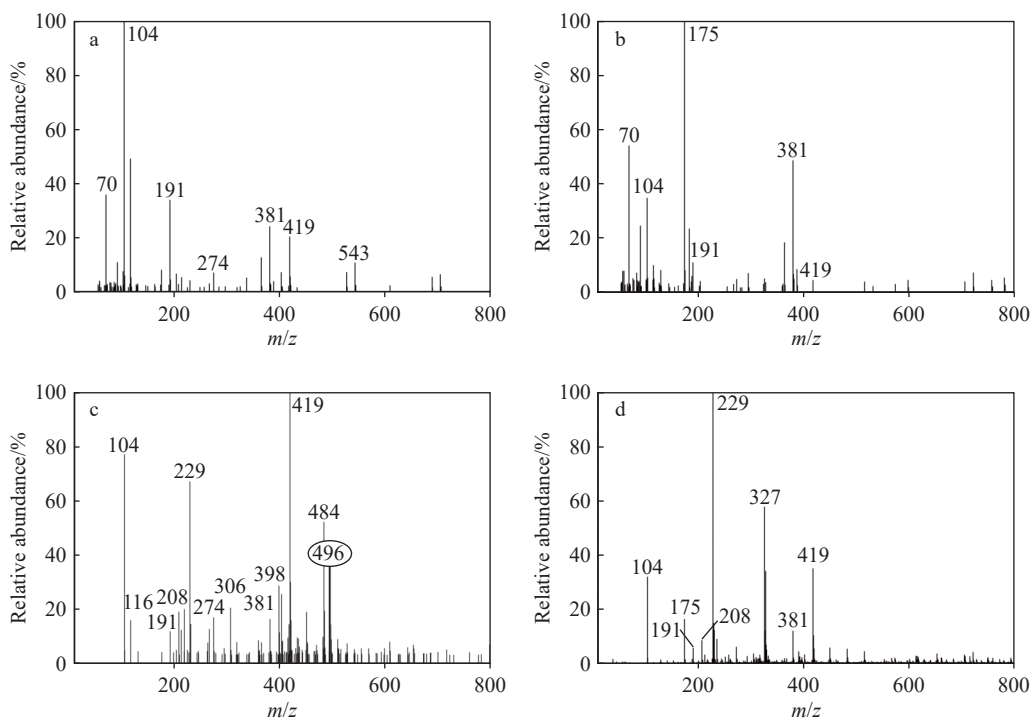
UENRT/CFI-MS 不仅具有 CFI-MS 的高灵敏度和高特异性, 还通过集成超声萃取和雾化技术实现了样品处理的革新, 简化了分析流程, 整个分析时间可缩短至几秒钟, 使得对整体样品的直接、快速分析成为可能, 为中药材研究开辟了新途径。



注: a. 欧当归; b. 当归(民乐); c. 当归(岷县); d. 当归(云南)

图2 当归和欧当归的 UENRT/CFI-MS 质谱图

Fig. 2 Mass spectra of *Angelica sinensis* and *Levisticum officinale* by UENRT/CFI-MS



注: a. 欧当归(ESI-MS); b. 岷县当归(ESI-MS); c. 欧当归(CFI-MS); d. 岷县当归(CFI-MS)

图3 样本提取液的 CFI-MS 和 ESI-MS 质谱图

Fig. 3 Mass spectra of sample extraction solution by CFI-MS and ESI-MS

### 3 结论

本研究采用 UENRT/CFI-MS 法快速鉴别当归和欧当归, 筛选出欧当归的  $m/z$  496 特征峰。该方法通过集成超声萃取、雾化和碳纤维离子化技术, 实现了从样品放置到获得质谱图仅需几秒钟的快速分析, 在复杂样品直接分析中具有优势, 有望在中药质量控制、快速筛查和其他相关领域得到广泛应用, 为提高中药材分析效率和准确性提供了有力工具。此外,  $m/z$  496 特征峰的结构有待进一步研究鉴定。

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