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Short communication

Simultaneous quantification of 16 bioactive constituents in *Common cnidium fruit* by liquid chromatography–electrospray ionization-mass spectrometry

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ABSTRACT

A novel quantitative method using high-performance liquid chromatography coupled to electrospray ionization tandem mass spectrometry was developed for simultaneous determination of the 16 important bioactive constituents including nine coumarins, and seven flavonoids in *Common cnidium fruit* samples from different regions. The separation was performed on a C_{18} column with linear gradient elution of acetonitrile and 0.1% acetic acid at a flow rate of 1.0 ml/min in 15 min. Quantification of the analytes was achieved by use of a hybrid quadrupole linear ion-trap mass spectrometer. Multiple-reaction monitoring scanning was employed with switching electrospray ion source polarity between positive and negative modes in a single run. The validation results of the method indicated that the method was simple, rapid, specific, and reliable. The results demonstrated that the quantitative difference in content of 16 bioactive constituents was useful not only for chemotaxonomy of many samples from different sources but also for the standardization and differentiation of many similar samples. Simultaneous quantification of bioactive components by high performance liquid chromatography–tandem mass spectrometric method would be a well acceptable strategy to comprehensively control the quality of *C. cnidium fruit*.

1. Introduction

Herbal drugs, individually and in combination, contain a myriad of compounds in complex matrices in which no single active constituent is responsible for the overall efficacy. Additionally, due to different sources and climates, the chemical constituents of a medicinal herb may vary substantially [1]. Simultaneous quantitative analysis of active components is the most direct and important method for the quality control of medicinal herbs [2].

Common cnidium fruit is the dried ripe fruit of *Cnidium monnieri* (L.) *Cusson*, *Apiaceae* and, as one of the most popular traditional Chinese medicinal herbs, it has been used to treat impotence, frigidity and skin diseases [3–5]. Phytochemical studies reveal that *C. cnidium fruit* contains many compounds such as coumarins, flavonoids, and volatile oil and their glycosides [6]. Until now, most of the established analytical methods such as high performance liquid chromatography–mass spectrometry (HPLC–MS) [6–9] and gas chromatography–mass spectrometry (GC–MS) [10] are capable of determining no more than five coumarin compounds in *C. cnidium fruit*. Recently, by coupling with mass spectrometry, Gao et al. and Chen et al. [6,7] detected and identified more than 16 bioactive components from the fruits of *C. monnieri*. However, the method was developed either only for qualitative characterization of coumarin and chromone constituents or quantification of only three major coumarins [6,7]. Therefore, it is still an analytical challenge for rapid analysis of more coumarin derivatives simultaneously due to their similar structures [4].

Liquid chromatography coupled with tandem mass spectrometry (LC–MS/MS) allows detection of trace amounts of constituents and has become an important technique for the analysis of the contents of medicinal herbs, in particular for low abundance compounds in complicated matrices which are difficult to obtain by conventional isolation means [11–14]. In this study, we developed

Abbreviations: CAD, collision activated dissociation; CE, collision energy; CXP, collision cell exit potential; DP, declustering potential; EP, entrance potential; EPI, enhanced product ion; IDA, information-dependent acquisition; MRM, multiple-reaction monitoring; TCM, traditional Chinese medicine.

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Table 1
HPLC–ESI-MS^n data of the 16 components from *Common cnidium fruit*.

Compounds	MW	t_R (min)	Ionization mode	MS_1 (m/z)	MS_2 (m/z)	DP (V)	CE (eV)
Osthole	244.3	9.93	ESI+	245.3[a]	189.1[a]	44	23
Xanthotoxol	202.2	5.56	ESI+	203.0[a]	147.1[a]	77	39
Isoporalen	186.2	7.78	ESI+	187.1[a]	131.1[a]	42	32
Isopimpinelline	246.2	8.11	ESI+	246.6[a]	232.0[a]	50	17
Bergapten	216.2	8.15	ESI+	217.0[a]	202.2[a]	60	30
Xanthotoxin	216.2	7.78	ESI+	217.0[a]	202.0[a]	60	30
Columbianedin	328.4	10.28	ESI+	329.0[a]	229.0[a]	47	13
Imperatorin	270.3	9.37	ESI+	271.0[a]	202.9[a]	50	16
Isoimperatorin	270.3	10.05	ESI+	271.0[a]	202.9[a]	50	16
Kaempferol	286.2	6.95	ESI−	284.7[a]	92.8[a]	−70	−50
Isorhamnetin	316.3	7.06	ESI−	314.8[a]	300.0[a]	−73	−33
Quercitrin	448.4	3.81	ESI−	446.8[a]	300.7[a]	−80	−34
Luteolin	286.2	5.65	ESI−	284.9[a]	132.9[a]	−75	−46
Quercetin	302.2	5.80	ESI−	300.7[a]	150.9[a]	−70	−30
Hesperidin	610.6	3.72	ESI−	608.8[a]	300.9[a]	−75	−25
Rutin	610.5	3.04	ESI−	608.8[a]	300.9[a]	−75	−25

[a] Monitored MRM transitions.

and validated a simple, sensitive and accurate HPLC–ESI-MS method for simultaneous determination of 16 bioactive constituents in *C. cnidium fruit*, including nine coumarins and seven flavonoids.

2. Materials and methods

2.1. Chemicals and samples

Acetonitrile and acetic acid were of HPLC-grade (Merck KGaA, Darmstadt, Germany). All other chemical solvents were of analytical grade (Beijing Chemical Factory, Beijing, China). The purity of compounds used in the current study is as follows: Osthole (1), 100.0%; xanthotoxol (2), 98.0%; isoporalen (3), 100.0%; isopimpinelline (4), 98.5%; bergapten (5), 98.1%; xanthotoxin (6), 98.0%; columbianedin (7), 98.0%; imperatorin (8), 99.5%; isoimperatorin (9), 99.6%; kaempferol (10), 93.2%; isorhamnetin (11), 99.0%; quercitrin (12), 99.3%; luteolin (13), 100.0%; quercetin (14), 97.3%; hesperidin (15), 100.0%; rutin (16), 100.0%. Twelve batches of *C. cnidium fruit* from six different regions in China were purchased from authenticated local pharmacies (SD-1, 2 and 3 from Shandong province, CZ-1, 2, and 3 from Hebei province, JS-1, 2, and 3 from Jiangsu province, HN from Henan province, ZJ from Zhejiang province and SC from Sichuan province), and further confirmed by professor Jiping Duan (Hebei Institute for Food and Drug Control, Shijiazhuang, China). All the voucher specimens were deposited in the department of pharmaceutical analysis, Hebei Medical University. The dry plant samples were ground to fine powder by a pulverizer, and 2.0 g of powder was accurately weighed and ultrasonically extracted with 30 mL of 70% ethanol for 60 min. The extracted solution was adjusted to the original weight by adding 70% ethanol, and then the aliquot of the supernatant was filtered through a 0.45 μm microporous membrane before HPLC.

2.2. Instrumentation and conditions

The LC system consisted of an Agilent Technologies Series 1200 system (Agilent, Cupertino, CA) equipped with an automatic degasser, a quaternary pump and an autosampler. Chromatographic separations were performed on a Waters XBridge C_{18} column (250 mm × 4.6 mm, 5 μm, Waters Corporation, Milford, MA) at 40 °C. The mobile phase consisted of acetonitrile (A) and water containing 0.1% acetic acid (B). The elution program was optimized as follows: 0–4.5 min, linear change from A–B (30:70, v/v) to A–B (50:50, v/v); 4.5–6.0 min, linear change from A–B (50:50, v/v) to A–B (80:20, v/v); 6.0–9.0 min, isocratic elution A–B (80:20, v/v); 9.0–10.0 min, linear change from A–B (80:20, v/v) to A–B (30:70, v/v); 10.0–15.0 min, isocratic elution A–B (30:70, v/v). This was followed by 5 min equilibration period prior to the injection of each sample. The flow rate was set at 1.0 mL/min. Aliquots of 5 μL were injected into the HPLC system for analysis.

For quantitative determination of the 16 compounds, all experiments were carried out in positive and negative mode simultaneously by using an Agilent Series 1200 (Agilent) liquid chromatography, equipped with a vacuum degasser, a binary pump, and an autosampler, connected to a Agilent ChemStation software and a 3200QTRAP™ system from Applied Biosystems/MDS Sciex (Applied Biosystems, Foster City, CA), a hybrid triple quadrupole linear ion trap mass spectrometer equipped with Turbo V sources and TurboIonspray interface.

The operating conditions for the ESI interface were as follows: the ion spray voltage was set to 4500 and −4500 V, respectively, the turbo spray temperature was maintained at 550 °C, nebulizer gas (gas1) and heater gas (gas2) was set at 50 and 60 arbitrary units, respectively. The curtain gas was kept at 25 arbitrary units and the interface heater was on. Entrance potential (EP) was set at 10/−10 V and collision cell exit potential (CXP) was set at 5/−5 V. Nitrogen was used in all cases. Multiple-reaction monitoring mode was employed for quantification. The dwell time of each ion pair was 40 ms. The full-scan mass covered the range from m/z 100 to 1000. All the peaks of target compounds in the solution of *C. cnidium fruit* samples were unambiguously identified by the comparison of retention time, parent and product ions with standards. The retention time, characteristic MS/MS fragmentation data, precursor-to-production pair, declustering potential (DP), and collision energy (CE) for each analyte are described in Table 1. All instrumentations were controlled and synchronized by Analyst software (version 1.4.2) from Applied Biosystems/MDSSciex.

2.3. LOD and LOQ

Preparation of standard solutions and calibration curves are described in Supplementary Methods. The working solutions were further diluted to a series of concentrations with methanol in order to determine the limits of detection (LOD) and quantification (LOQ). The LOD and LOQ under the prevalent chromatographic conditions were defined as the analyte mass resulting in a signal-to-noise (S/N) ratio of 3 and 10, respectively.

2.4. Precision, repeatability and stability

The intra-day variation was determined by analyzing the six replicates on the same day and the inter-day variation was determined on 3 consecutive days. The relative standard deviation (RSD)