RAPID QUALITATIVE AND QUANTITATIVE ANALYSES OF TEN ACTIVE COMPONENTS FROM BUFEIYISHEN BY UPLC-Q-ORBITRAP MS

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ABSTRACT

Introduction*: Bufei Yishen formula (BYF) has been extensively applied in clinical treatment of chronic obstructive pulmonary disease (COPD) and provides an effective treatment strategy for the syndrome of lung-kidney qi deficiency in COPD patients. The aim of our study is to establish a rapid and integrated method for rapid qualitative and quantitative analyses of active compounds from bufeiyishen by UPLC-quadrupole-Orbi- trap mass spectrometry(UPLC-Q-Orbitrap MS).*

Materials and methods*: UPLC-Q-Orbitrap MS method was used to establish a rapid method for simultaneous identification and quatification of active components in BYF. The confirmed method was applied to the analysis of active components in BYF.*

Results*: A total of 10 active compounds were identified unambiguously by comparing the retention times and the exact m/z ratio in the full scan MS mode of the authentic standards with the sample. This method was validated by LODs, LOQs, precision, repeatability, stability, mean recovery, recovery range and RSD. The results indicate that all of the analytes in the sample solution were stable for 12 h.*

Discussion*: The UPLC-MS/MS method was simple, fast, and showed good linearity, precision, and recovery for determination of the 10 major constituents. Furthermore, the modified UPLC-MS/MS method was applied for the quality evaluation of the quantitative determination of 10 compounds could be suitable for the quality control of three batches of BYF samples, which will facilitate its clinical usage and quality control.*

Keywords*: Bufei Yishen formula, active components, quantitative analyses, UPLC-Q-Orbitrap MS.*

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Introduction

BufeiYishen formula (patent: ZL.201110117578), including twelve medicinal herbs, is specially prescribed for chronic obstructive pulmonary disease (COPD) patients with lung-kidney qi deficiency syndrome[1]. Previous studies demonstrated that BYF had the potential to alleviate COPD symptoms by reducing the exacerbation frequency, delaying acute exacerbation, and improving pulmonary function and exercise capacity of COPD patients[2-4].

System biology analysis of anti-COPD mechanisms of BYF showed that BYF achieved its ameliorative effect over COPD probably by regulating immune response, inflammatory response, lipid metabolism, etc[5,6]. However, little is known about the qualitative and quantitative analyses of the active compounds in BYF.

In recent years, the LC-MS/MS method has become the most powerful tool for quantitative analysis of plant constituents due to its high sensitivity, selectivity, and easy maneuverability[7]. Orbitrap is the newest instrument for high-resolu-

tion mass spectrometry (HRMS), which is capable of reaching a resolving power in excess of 10,00,000 (FWHM, full width half maximum)[8]. It was proved that higher mass resolution can provide improved selectivity in complex sample matrices[9]. Moreover, High resolution hybrid quadrupole-Orbitrap mass spectrometry (Q-Orbitrap MS) technology[10] offers advantages in analytical sensitivity and specificity over other techniques and can also simultaneously perform qualitative and quantitative analyses.

In the present work, we established a rapid and integrated method for Rapid qualitative and quantitative analyses of active components compounds from bufeiyishen by UPLC-Q-Orbitrap MS.

Material and methods

HPLC-grade acetonitrile, formic acid, and methanol were obtained fromFisher Scientific (Loughborough, UK). Water was purified by using a Milli-Q system (Millipore, Billerica, MA, USA). Ethanol was purchased from Beijing Chemical Reagent Co., Ltd. (Beijing, China). Reference standards of ginsenoside Re, ginsenoside Rb1, ginsenoside Rg1, Astragaloside IV, Peimine, Schisandrin B, Icariin, Hesperidin,Naringin, Paeoniflorin were purchased from the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). All standards were of at least 98% purity and were suitable for UHPLC-LTQ-Orbitrap analysis. Three batches of BYF (Med-drug Permit No. 20180426, 20180508 and 20180603) were obtained from The First Affiliated Hospital of Henan University of Traditional Chinese Medicine(Zhengzhou, China)

The powder of 1g BYF granule was accurately weighed and extracted with 500 mL 50% methanol in an ultrasonic water bath for 60 min at room temperature, and then centrifuged at 12000 r/min for 10 min to get the supernatant. Respective standard stock solutions of 10 components (ginsenoside Re, ginsenoside Rb1, ginsenoside Rg1, Astragaloside IV, Peimine, Schisandrin B, Icariin, Hesperidin, Naringin and Paeoniflorin) were prepared at concentrations of 50 ng/mL by weighing the desired amount of each component into a volumetric flask and dissolving it in 50% methanol. All the samples were filtered through 0.22μm nylon membrane filters and analyzed directly by UHPLC-LTQ-Orbitrap.

Sample analyses were performed on a Thermo UHPLC system (Thermo Fisher Scientific, San Jose, CA, USA) equipped with a binary pump, an online degasser, a thermostated autosampler, a thermostatically controlled column compartment, and a diode array detector (DAD). The auxiliary gas and sheath gas were set to flow rates of 10 and 35(arbitrary units)bar, respectively; capillary temperature, 350°C. The auxiliary gas temperature, 200°C, spray voltage, 3.5 kV; tube lens voltage, 120 V. The resolution was 70,000 (FWHM at 200 m/z). The automatic gain control target was set at 3.0e6 with a maximum injection time of 200 ms. The full MS scan ranges were set from 100 to 1500 m/z. Samples were analyzed in the positive and negative ion modes, separately.

Chromatographic separation was performed on a reverse-phase column Phenomenex kinetex C18 column C18 column (2.1 × 100 mm, 2.6μm) maintained at 25°C. The mobile phases A and B comprised 1‰ formic acid in water and acetonitrile, respectively. The following linear gradient was used: 0.0-2.0 min: 5% B; 2.0-12.0 min: 5-100% B;12.0-15.0 min:100% B. The flow rate was set to 0.3 mL/min, and the injection volume was 5μl. The on-line UV spectra were recorded in the range of 200-400 nm.

The UHPLC-Q-Orbitrap MS was validated with respect to linearity, sensitivity, accuracy, stability, precision, reproducibility and recovery.

Determination of linearity, LOD and LOQ

Calibration curves were established through the peak area of each authentic standard based on the least-squares linear regression model. The linearity of all calibration curves was evaluated by the correlation coefficients. For each authentic standard, the limit of detection (LOD) is defined as the concentration of the required authentic standard which can produce a signal equal to the background plus three times the standard deviation of the blank. The limit of quantification (LOQ) was estimated by injecting a series of increasingly dilute standard solutions until the signal-to-noise ratio decreased to 1011.

Reproducibility and stability

The precision was evaluated by intra-day and inter-day variability. The relative standard deviations (RSDs) were used as an indicator of precision. The accuracy of this method was determined by the recovery test. The samples were spiked with one concentration level of the known amounts of 10

authentic standards. The spiked samples of the concentration were analyzed in triplicate. The recovery was determined by comparing the amount of the analytes added to the sample detected by UHPLC-Q-Orbitrap MS. The RSDs were used in the evaluation of the reproducibility and the acceptance criterion was a maximum of 5.0% deviation. The stability of the sample solution and standard solutions (stored at 4 °C) were tested at room temperature on three consecutive days. Injections were performed at 0, 2, 4, 8, 12 h. The variations were expressed as relative standard deviations.

Results and discussion

Method validation

All of the 10 authentic standards were indicated in Table 1.

No	Name	Rt (min)	Formula	Ion	Measured (M/Z)	Theoretical (M/Z)	MS/MS	Error (ppm)
1	Paeoniflorin	5.77	$C_{23}H_{28}O_{11}$	[M-H]$^-$	479.1704	479.1723	182.0218	-0.938
2	Peimine	6.11	$C_{27}H_{45}NO_3$	[M+H]+	432.3471	432.3472	414.3453	-0.019
3	Hesperidin	6.45	$C_{28}H_{34}O_{15}$	[M-H]$^-$	609.1971	609.1975	367.2421	-0.567
4	Ginsenoside Re	6.62	$C_{48}H_{82}O_{18}$	[M-H]$^-$	945.5428	945.5428	991.5425	1.119
5	Ginsenoside Rg_1	6.92	$C_{42}H_{72}O_{14}$	[M+HCOO]-	799.4847	799.4849	845.4849	0.209
6	Icariin	7.18	$C_{33}H_{40}O_{15}$	[M-H]$^-$	675.2442	675.2441	367.2541	0.005
7	Ginsenoside Rb_1	7.49	$C_{54}H_{92}O_{23}$	[M-H]$^-$	1107.5431	1107.5957	1107.5957	-0.059
8	Astragaloside IV	7.93	$C_{41}H_{68}O_{14}$	[M+HCOO]-	783.4537	783.4536	829.1421	1.49
9	Naringin	8.93	$C_{27}H_{32}O_{14}$	[M+H]+	581.535	581.5275	419.0499	3.35
10	Schisandrin B	11.93	$C_{23}H_{28}O_6$	[M-H]$^-$	399.1959	399.1959	367.5468	0.087

Table 1: The performance characteristics of 10 authentic standards.

No	Name	Linear regression			LOD	LOQ	Precision RSD (%)	Repeatability RSD (%)	Stability	Mean recover(%)	Recovery range (%)	RSD(%)
		Linear range(mg.L^{-1})	Regression equation	r	(μg·L^{-1})	(μg·L^{-1})						
1	Paeoniflorin	0.212—8.480	$Y=1.02392\times10^7+9.07286\times10^4X$	0.9983	1.46	4.93	2.2	1.41	2.2	98.02	97.3-99.7	2.7
2	Peimine	0.019—1.248	$Y=6.90566\times10^6+6.86979\times10^4X$	0.9996	1.62	5.36	2.2	2.01	2.2	100.01	98.9-102.4	2.8
3	Hesperidin	0.216—8.640	$Y=1.39158\times10^7+6.3105\times10^3X$	0.9991	2.08	6.9	2.5	1.53	1.9	98.69	97.5-99.2	3.6
4	Ginsenoside Re	0.016—1.040	$Y=97377.9+9.09222\times10^3X$	0.9989	2.33	7.49	2.5	1.16	2.7	99.21	98.2-99.5	2.1
5	Ginsenoside Rg_1	0.019—1.220	$Y=1.03762\times10^6+1.36804\times10^4X$	0.9995	1.81	6.57	2.2	1.87	2.5	99.99	98.9-100.5	3.1
6	Icariin	0.018—1.120	$Y=-295161+2.10723\times10^4X$	0.9996	1.92	6.98	2.4	1.93	2.4	98.94	98.9-99.5	2.4
7	Ginsenoside Rb_1	0.022—1.391	$Y=377522+3.02791\times10^3X$	0.9991	3.33	11.6	1.9	1.16	1.8	97.65	95.6-98.1	2.6
8	Astragaloside IV	0.016—1.024	$Y=1.55315\times10^6+1.98695\times10^4X$	0.9996	1.48	5.16	2.3	1.23	2.8	99.78	98.1-99.9	3.4
9	Naringin	0.043—1.359	$Y=4.2302\times10^7+7.47691\times10^4X$	0.9991	1.47	4.89	1.7	1.58	2.3	98.65	98.1-100.2	4.1
10	Schisandrin B	0.020—1.263	$Y=6.52761\times10^6+5.53307\times10^4X$	0.9997	1.32	4.72	2.2	1.15	2.5	98.84	98.8-100.8	2.1

Table 2: Summary of calibration parameters, LOD, LOQ, precision, repeatability, stability, mean recovery, recovery range and RSD for the 10 authentic standards (n = 3).

All calibration curves of the 10 analytes and their performance characteristics are presented in Table 2. The correlation coefficients of all of the standards were more than 0.99, which showed that all calibration curves were linear over the entire calibration range. The LOD and LOQ ranged from 1.32 to 3.33 μg/L and from 4.72 to 11.6 μg/L. respectively (Table 2), demonstrating that the chosen method was sensitive enough to detect the trace constituents in BYF.The precision, reproducibility, stability and accuracy of the 10 analytes are summarized in Table 2. The RSD values of the precision and Stability ranged from 1.7% to 2.5% and from 1.8% to 2.8%, respectively.which indicates that all of the analytes in the sample solution were stable for 12 h.

The recoveries of the 10 analytes ranged from 97.5% to 102.6%. The mean recoveries were all above 97.65% and below100.01% with RSDs less than 2.1%.

These results show that the established method has good linearity, precision, reproducibility, stability, accuracy, and sensitivity for all compounds in the simultaneous assay of the 10 compounds studied in BYF.

Identification of active compounds in BYF

We established that the rapid and validated analytical method by UHPLC-Q-Orbitrap MS was efficient for the quantification of the 10 active compounds in BYF. Fig. 1 shows the extracted ion chromatograms of mixed standards of the 10 compounds in BYF under the optimal UPLC-Q-Orbitrap MS conditions. The content of each com-

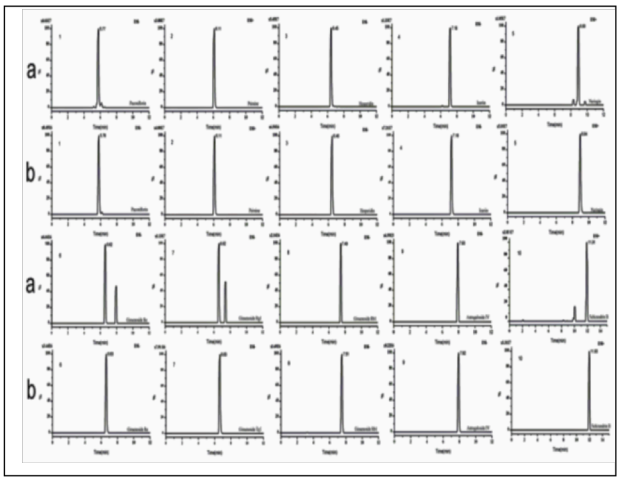

Fig. 1: The ion chromatograms of mixed standards of 10 authentic standards under theoptimalUHPLC-Q-Orbi-MScondition. 1 Paeoniflorin, 2 Peimine, 3 Hesperidin, 4 Icariin, 5 Naringin, 6 Ginsenoside Re, 7 Ginsenoside Rg1.

pound was calculated by the respective calibration curve, and the quantification results from three parallel determinations are shown in Table 3. The total ion chromatograms (Fig. 2-3) provide a relatively integrated frame of the metabolomic analysis.

	Batch			Average
component	1	2	3	
Paeoniflorin	4.188	4.327	4.061	4.192
Peimine	0.284	0.294	0.264	0.281
Hesperidin	6.465	6.285	6.51	6.42
Ginsenoside Re	0.555	0.591	0.579	0.575
Ginsenoside Rg1	0.463	0.472	0.439	0.458
Icariin	0.321	0.327	0.362	0.337
Ginsenoside Rb1	0.542	0.55	0.534	0.542
Astragaloside IV	0.022	0.025	0.02	0.022
Naringin	0.106	0.112	0.109	0.109
Schisandrin B	0.286	0.277	0.295	0.286

Table 3: Contents of 10 compounds in BYF (mg/g, n = 3).

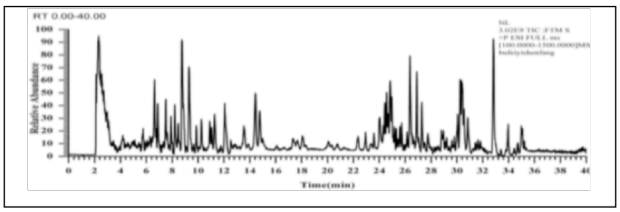

Fig. 2: Total ion chromatogram (TIC) of BYF in positive ion mode.

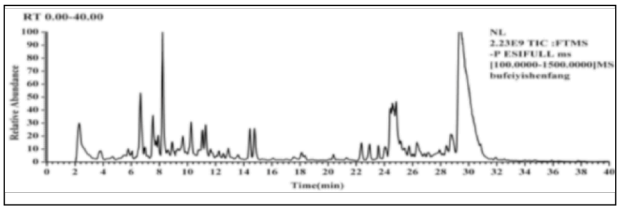

Fig. 3: Total ion chromatogram (TIC) of BYF in negative ion mode.

A total of 10 active compounds were identified unambiguously by comparing the retention times and the exact m/z ratio in the full scan MS mode of the authentic standards with the sample. The Q-Orbitrap MS, a powerful high-resolution mass spectrometer, has the capability of simultaneous qualitative and quantitative analysis.

The method was successfully applied to quantify the contents of the 10 major constituents in BYF. the content of Hesperidin was the highest, Astragaloside IV was lowest. This method may lay foundation for the quality control of BYF.

Conclusion

In this study, a method based on UPLC–Q-Orbitrap MS was established for identifying multiple components in BYF. A UPLC-MS/MS method was modified for simultaneous determination of 10 active compounds in BYF. The UPLC-MS/MS method was simple, fast, good linearity, precision and recovery for the determination of 10 major constituents. Furthermore, the modified UPLC-MS/MS method was applied for the quality evaluation of the three batches of BYF samples. The quantitative determination of 10 compounds could be suitable for the quality control of BYF. We expect that all these experiments would be helpful for better comprehension of the pharmacodynamic profile and in vivo absorption situation of BYF, which will facilitate its clinical usage and quality control.

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