Simultaneous Quantification of 10 Bioactive Polyphenolic Compounds in Menerba by a High Throughput LC-MS/MS

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OVERVIEW

NOVEL ASPECT
Simultaneous quantification of multiple bioactive polyphenolic compounds in herbal matrices with a high throughput LC-MS/MS method.

PURPOSE
To develop an automated, simple and fast liquid chromatography-tandem mass spectrometry platform for the simultaneous quantification of natural bioactive polyphenolic compounds in Menerba.
To validate the method in herbal extract matrices following FDA guidelines, using LC-MS/MS.
To quantify multiple actives in herbal extracts and production samples.

METHODS
Using LC-MS/MS in combination with automated online sample preparation (LC/LC-MS/MS) to quantify trace amounts of bioactive polyphenolic compounds.

RESULTS
The simultaneous quantification of multiple bioactive polyphenolic compounds extracted from Chinese herbs using a high-throughput and sensitive LC-MS/MS method was developed and validated in herbal matrix. The recoveries of extraction from herbal matrix were > 80 %. All actives in the extraction solution were stable for at least 4 days when stored at 4°C, allowing for sufficient time for analysis without degradation. The method had a range of reliable response from 0.2 to 1500 ng/mL for all analytes. The lower limits of quantitation (LLOQ) range were from 0.2 to 63 ng/mL. The intra-day and inter-day accuracies for the method were 94.4-111.1 %, and the inter day precisions were < 11.8 % (coefficients of variance as assessed over 3 days at 3 different concentrations: low, medium and high).
The assay met all predefined acceptance criteria and proved to be suitable for quantitative analysis of herbal extracts as well as for formulation studies.

INTRODUCTION

Menerba is an oral botanical drug designed for the safe and effective treatment of vasomotor symptoms (hot flashes) associated with menopause. It is a selective estrogen receptor beta modulator, which is associated with inhibition of menopausal hot flashes. Menerba is extracted from 22 Chinese botanical species containing flavones, isoflavones, chalcones, and norligans. Many of these polyphenolic compounds demonstrate biological and medicinal properties such as anti-inflammatory, antioxidant activity, anticancer, antibacterial, antiviral, antimutagenic effects and anticarcinogenic effects. Our goal was to develop a high-throughput sensitive LC-MS/MS method for the simultaneous quantification of trace amounts of multiple polyphenolic compounds in Menerba.

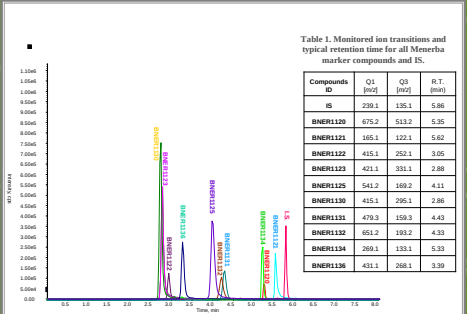


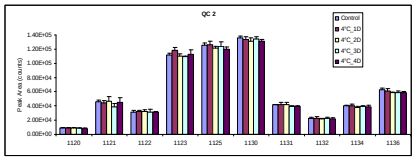
Table 1. Monitored ion transitions and typical retention time for all Menerba marker compounds and IS.

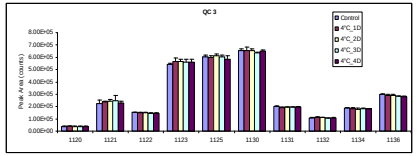
Compounds ID	Q1 [m/z]	Q3 [m/z]	R.T. (min)
IS	239.1	135.1	5.86
BNER1120	675.2	513.2	5.35
BNER1121	165.1	122.1	5.62
BNER1122	415.1	252.1	3.05
BNER1123	421.1	331.1	2.88
BNER1125	541.2	169.2	4.11
BNER1130	415.1	295.1	2.86
BNER1131	479.3	159.3	4.43
BNER1132	651.2	193.2	4.33
BNER1134	269.1	133.1	5.33
BNER1136	431.1	268.1	3.39

Figure 1. Representative ion chromatogram of a standard mixture containing all Menerba marker compounds and internal standard (IS).

Table 2. Analysis linearity range, regression coefficient (r), LOD, and LLOQ for 10 actives in herbal matrices (n = 3).

Compound ID	Linearity Range (ng/ml)		r	LLOQ	S/N	LOD	S/N
	LLOQ	ULOQ		(ng/ml)	at LLOQ	(ng/ml)	at LOD
BNER1120	0.783	50.1	0.9986	0.783	10	0.196	3.2
BNER1121	62.7	1500	0.9988	62.7	13.4	31.3	4.1
BNER1122	0.781	37.5	0.9990	0.781	16.3	0.195	3
BNER1123	0.781	50	0.9981	0.781	44.2	0.0651	5.8
BNER1125	0.391	25.1	0.9982	0.391	23.5	0.0326	3.1
BNER1130	0.197	50.5	0.9992	0.197	21.3	0.0658	4.7
BNER1131	3.91	250	0.9981	3.91	41	1.95	5.2
BNER1132	1.95	125	0.9994	1.95	27.5	0.163	4.5
BNER1134	0.313	10	0.9996	0.313	15.8	0.0781	5
BNER1136	0.391	50	0.9990	0.391	12.2	0.0651	5.8





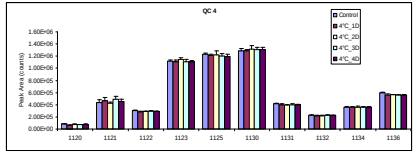


Figure 2. Stability of 10 actives in extraction solution for 1-4days (4 ℃) at low (QC2), medium (QC3), and high (QC4) concentration levels (n = 4).

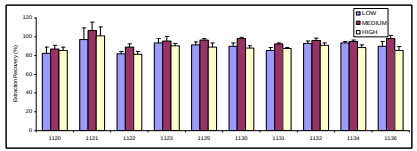


Figure 3. Extraction recoveries from herbal matrix at low, medium, and high concentration levels (n = 4).

EXPERIMENTAL

Sample Preparation and Instrumentation:

Freeze dried Menerba powder drug substance was weighed out and mixed with an internal standard (2', 4'-dihydroxychalcone) solution (methanol/water/formic acid, 80/19.9/0.1, v/v) on a vortexer for 15 min (room temperature, 1,500 rpm). The above solution (10 mg/mL) was then centrifuged for 15 min (4˚C, 13,000 rpm). The appropriate dilutions were made from the supernatant and transferred to HPLC vials for analysis.

The calibrators and QCs were prepared from serial dilution of the standard working solution, which contained 10 Menerba marker compounds (BNER1120, BNER1121, BNER1122, BNER1123, BNER1125, BNER1130, BNER1131, BNER1132, BNER1134, and BNER1136), with the same internal standard solution.

Two Agilent 1200 HPLC systems (consisting of two degassers, two binary pumps, one column switching valve and one column compartment), and one Leap Technologies HTS PAL autosampler were used for sample extraction and separation. After sample injection (20 µL), all analytes were chromatographed on an Agilent Zorbax Eclipse XDB-C8 column (150 mm x 4.6 mm, 5 um). Mobile phases were composed of aqueous formic acid (0.1%, v/v) and methanol with a flow rate of 0.8 mL/min. Samples were first loaded onto an online extraction column (4.6 × 12.5 mm, Eclipse XDB-C8) and were washed (1.5 mL/min) for 30s. Then the switching valve was activated and the analytes were backflushed onto the C8 analytical column (4.6 × 150 mm, Eclipse XDB-C8). The linear gradient was started with 50% methanol (v/v) and ramped to 100% methanol within 1.5 min and the total run was 8 min. The analytes were quantified using an API5000 MS/MS system (Sciex/ Applied Biosystems) in ESI negative MRM mode. The monitored ion transitions and typical retention times are shown in Table 1.

RESULTS & CONCLUSIONS

The simultaneous quantification of 10 bioactive polyphenolic compounds extracted from Menerba using a high-throughput and sensitive LC-MS/MS method was developed and validated in Menerba herbal matrix. Figure 1 is an LC-MS/MS chromatogram of all actives and internal standard. No ion suppression interfered with any of the signals of compounds of interest.

The stock working solution is stable at room temperature for at least 24 h with acceptable accuracies (93.2-106.0%). All actives in the extraction solution were stable for at least 24 h when stored at room temperature and 4°C (e.g., in the autosampler) (Figure 2); this allows for sufficient time of analysis without degradation. The extraction procedure was systematically developed and the conditions described above gave the best results. The extraction recoveries of all compounds from herbal matrix were above 80% (Figure 3). No interfering peaks were found at the retention times of the analytes.

The method had a linear range of reliable response from 0.2 to 200 ng/mL for most analytes. The lower limits of quantitation (LLOQ) range on column were from 0.004-1.25 ng. BNER1125, BNER1133, BNER1134, and BNER1136 were 0.008 ng on column. BNER1120, BNER1122, and BNER1123 had LLOQs of 0.016 ng on column, BNER1130 had LLOQs on column of 0.004 ng, BNER1132 had 0.04 ng on column, BNER1131 and BNER1121 had LLOQs of 0.078 and 1.25 ng on column, respectively. The linear range was determined by regression coefficients (r) of greater than 0.998. The typical standard curve parameters for 10 actives in herbal matrices show in Table 2. The intra-day and inter-day accuracies for the method were 94.4-111.1%, with the precisions were <11.8 % (coefficients of variance were assessed over 3 days at 3 different concentrations).

In summary, the development and validation of an LC/LC-MS/MS assay for the quantification of BNER1120, BNER1121, BNER1122, BNER1123, BNER1125, BNER1130, BNER1131, BNER1132, BNER1134, and BNER1136 in herbal extracts and production samples met all pre-defined acceptance criteria.