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Development of High Performance Liquid Chromatography Tandem Mass Spectrometry Method for Quantitative Analysis of Bacopaside-I in Rat Urine and Feces Samples

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Introduction

Bacopaside I ($C_{46}H_{74}O_{20}S$) is one of active components isolated from Bacopa monnieri (L.) Wettst. (Brahmi), which has been used as an Ayurvedic medicine plant for centuries [1]. It has been reported that Brahmi has potential therapeutic effect in treatment and prevention of neurological diseases and improvement of cognitive processes[2,3]. Memory enhancing effect of Brahmi has been established through animal experiments and in healthy volunteers. The damarane triterpenoid saponin, Bacopaside I, is a main active compound in Brahmi. Reversed phase HPLC method has been established and used to determine bacopaside I and other active components in the plant extracts as well as biological samples[4]. However, so far LC/MS method for quantitative analysis of Bacopaside I in biological samples has not been reported. We report for the first time the development and validation of a LCMS/ MS method, aiming for pharmacokinetic study of bacopasides I.

Experimental

A LCMS-8030 (Shimadzu Corporation, Japan) triple quadrupole LC-MS/MS system was used in this method development study. A fast gradient elution separation program using a Kinetex C18 HUPLC column (1.7 um, 50 mmL × 2.1 mmID) was developed and optimized. The MRM transitions employed were 979.4>473.4 for bacopaside I and 609.3>195.0 for reserpine as internal standard (IS) in ESI positive mode. Rat urine and feces were used as biological matrix in the development and validation of a quantitative analysis method for bacopaside I. Liquid-liquid extraction method was employed in sample extraction and purification.

Table 1 LCMS-8030 conditions for quantitative analysis of Bacopaside-I

LC conditions	
Column	Kinetex C18 column (1.7 um), 50 mm L × 2.1mm ID
Mobile phase	A: Water (0.1% formic acid) B: Acetonitrile (0.1% formic acid)
Elution mode	Gradient elution 10 minutes
Flow rate	0.3 mL/min
Column temp.	40°C
Injection vol.	15 uL

MS conditions & MRM parameters	
Interface	ESI
MRM parameters (positive)	Bacopaside I: 979.4>483.4, CE:-26V, Q1B: -25V; Q3B: -25V Reserpine (IS): 609.3>195.0 CE:-37V
Block temperature	300°C
CDL temperature	250°C
Nebulizing gas flow	Nitrogen, 2.0 L/min
Drying gas flow	Nitrogen, 15 L/min

Results and Discussion

1. Method development: Bacopaside I is ionized by ESI in both positive and negative modes to form $[M+H]^+$ (*m/z* 979.4) and $[M-H]^-$ (*m/z* 977.4). We have established an LCMS-IT-TOF method based on negative ESI mode due to its higher intensity in MS mode [5]. It was found that for MRM method, positive ESI method (979.4>483.4) showed better sensitivity than negative mode. The structure and fragmentation of bacopaside I under MRM conditions are shown Fig 1.

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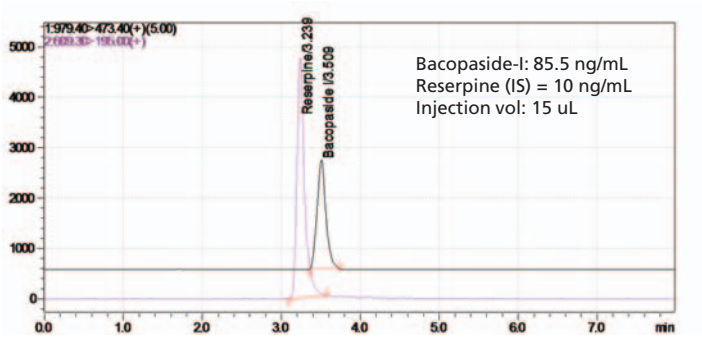
Fig. 2 shows the chromatograms of a mixed sample of bacopaside I and reserpine. It can be seen that reserpine is an ideal internal standard (IS) in this method. The elution of the two compounds were closed, but not overlapped. Therefore, ion suppression of the IS to bacopaside I was avoided.



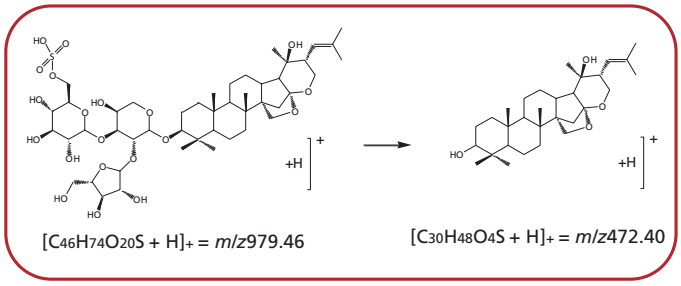


Fig 1. Structure and Fragmentation pathway of Bacopaside-I on LC/MS/MS in positive mode.

Fig 2. MRM chromatograms of Bacopaside-I and IS on LCMS-8030

2. Performance evaluation: The performance of the MRM quantitation method was evaluated systemically using spiked samples prepared from rat urine and feces by LL extraction method. The quantitation calibration curve of bacopaside I is shown in Fig. 2. The linearity (r^2) was 0.9999 for a range from 8.6 ng/mL to 900 ng/mL.

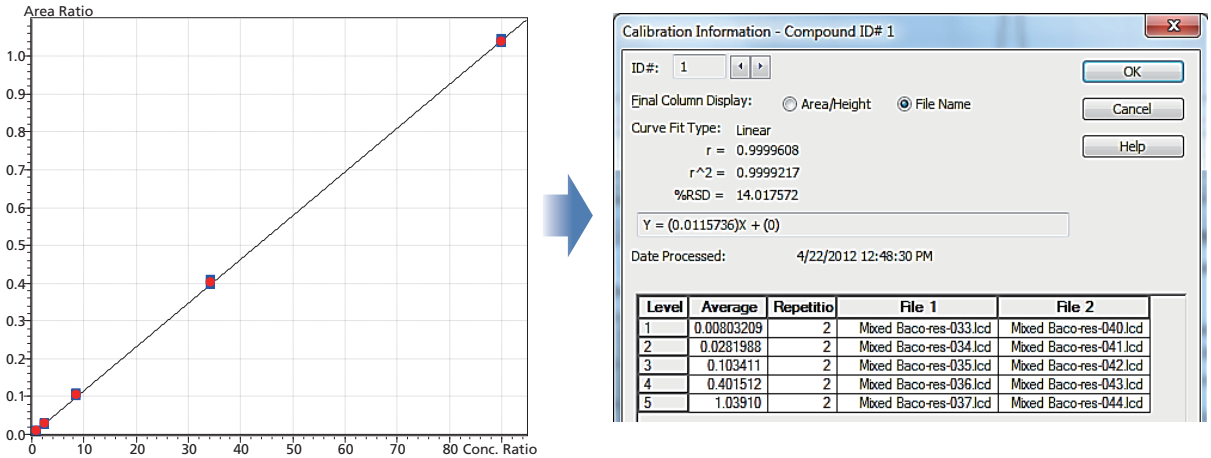


Fig 3. Calibration curve of Bacopaside-I by internal standard method on LCMS-8030. Conc.of bacopaside I: 8.6, 25.6, 85.5, 342 and 900 ng/mL (IS: 10 ng/mL).

Due to extremely flat baseline, the LOD and LOQ could not be calculated from S/N ratio. The lowest concentration of bacopaside I detectible by this method was about 4.3 ng/mL (spiked in urine) with poorer reproducibility (Fig. 4). The peak area reproducibility at 8.6, 25.7 and 85.5 ng/mL (spiked in urine) was 17.2%, 6.3% and 2.9% (RSD, n=6), respectively. Based on these results, the LOQ of the method is estimated to be about 15 ng/min and LOD is about 5 ng/mL. The recoveries of bacopaside I in urine (spiked) were between 104% and 129% for three concentration levels (45, 72 and 90 ng/mL) (Table 2) . However, the recovery

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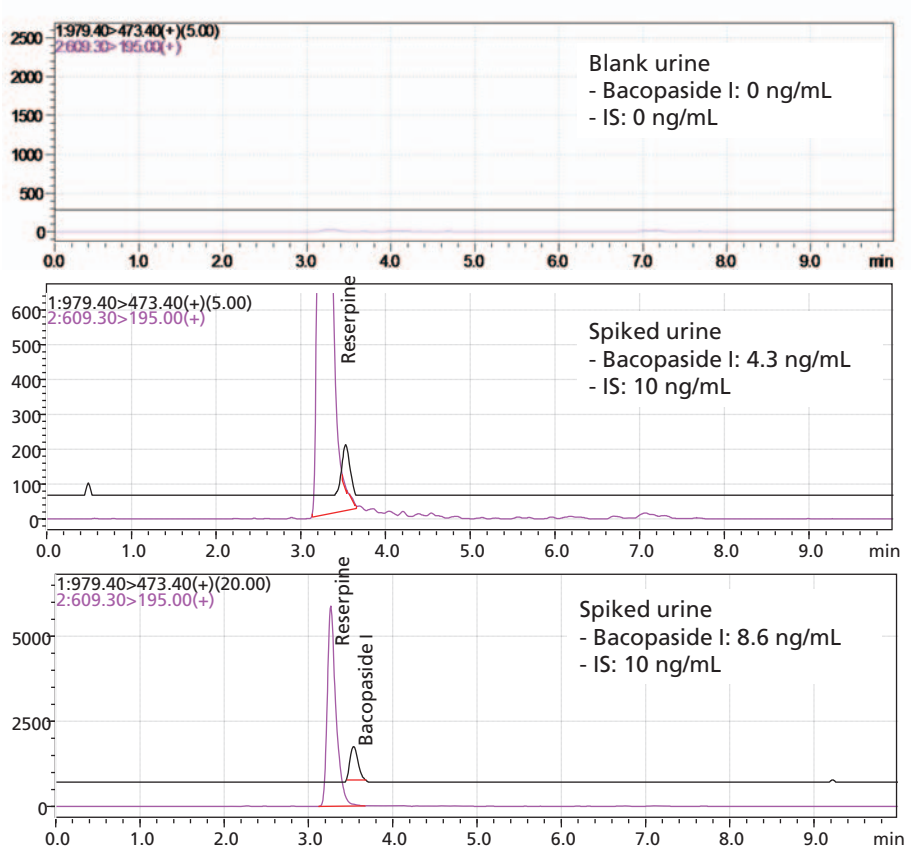

Fig 4. Chromatograms of urine blank and spiked standard samples in urine.

Table 2 Recovery of Bacopaside I in urine samples

No	Sample Name	Conc. Spiked (ng/mL)	Ret. Time (min)	Conc. (ng/mL)	Recovery (%)	Ave Recovery (%)
1	3/1 urine n1	45	3.532	45.42	100.9	103.6
2	3/2 urine n2	45	3.534	45.36	100.8	
3	3/3 urine n3	45	3.531	49.12	109.2	
4	3/4 urine n1	72	3.531	75.48	104.8	115.2
5	3/5 urine n2	72	3.534	87.12	121.0	
6	3/6 urine n3	72	3.535	86.24	119.8	
7	3/7 urine n1	90	3.529	116.52	129.5	129.3
8	3/8 urine n2	90	3.545	108.73	120.8	
9	3/9 urine n3	90	3.534	123.77	137.5	

Table 3 Recovery of Bacopaside I in feces samples

No	Sample Name	Conc. Spiked (ng/mL)	Ret. Time (min)	Conc. (ng/mL)	Recovery (%)	Ave Recovery (%)
1	3/1 feces n1	45	3.519	20.37	45.3	42.1
2	3/2 feces n2	45	3.515	15.8	35.1	
3	3/3 feces n3	45	3.522	20.65	45.9	
4	3/4 feces n1	72	3.504	32.71	45.4	44.4
5	3/5 feces n2	72	3.501	32.38	45.0	
6	3/6 feces n3	72	3.506	30.9	42.9	
7	3/7 feces n1	90	3.511	26.58	29.5	41.2
8	3/8 feces n2	90	3.502	46.41	51.6	
9	3/9 feces n3	90	3.503	38.37	42.6	

Conclusions

For the first time, a high sensitivity LC/MS/MS method for quantitative analysis of bacopaside I in rat urine and feces has been developed and validated. The LOD and LOQ of the method were estimated to be 5 and 15 ng/mL. The recovery of the method for urine samples was at 104~129%, but much lower for feces samples. Further study to improve extraction recovery from feces sample is undergoing.

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