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Qualitative and Quantitative Analysis of Seven Signature Components in the Fruiting Body of *Antrodia cinnamomea* by HPLC–ESI–MS/MS

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Summary. An accurate and rapid liquid chromatography–electrospray ionizaion–tandem mass spectrometry (LC–ESI–MS/MS) analytical method was developed and validated for the simultaneous determination of antcins A, B, C, H, and K, dehydroeburicoic acid, and 4,7-dimethoxy-5-methyl-1,3-benzodioxole in the extract and capsule of *Antrodia cinnamomea* (AC) fruiting body. These seven signature compounds were ionized using an electrospray ion source and analyzed by a triple-quadrupole mass analyzer under a multiple reaction monitoring (MRM) mode. The MRM transitions of m/z 453/409 (antcin A), m/z 467/408 (antcin B), m/z 469/425 (antcin C), m/z 485/413 (antcin H), m/z 487/407 (antcin K), m/z 467/337 (dehydroeburicoic acid), and m/z 197/139 (4,7-dimethoxy-5-methyl-1,3-benzodioxole) were used to quantify these seven components, respectively. Their calibration curves presented good linear regressions ($R^2 > 0.997$) within the tested concentration range. The intra- and inter-day precisions were less than 1.97% and 2.53%, respectively. The overall recovery was in the range of 87.55%–95.41%. This validated high-performance liquid chromatography (HPLC)–MS/MS method offers promising applications for the accurate and rapid quantification of signature compounds in the fruiting body and its commercial products.

Key Words: *Antrodia cinnamomea*, multiple reaction monitoring (MRM), tandem mass spectrometry, triterpenoid

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Introduction

Antrodia cinnamomea (AC; synonyms: *Antrodia camphorata* and *Taiwanofungus camphoratus*, Polyporaceae, Aphyllophorales) is a rare and valuable medical fungus in Taiwan [1]. The fruiting body of AC growing restrictedly on the inner cavity of the rare and endangered species *Cinnamomum kanehirai* Hayata is a costly folk remedy for treating various diseases including liver diseases, hypertension, abdominal pain, and cancer in Taiwan [2]. The isolated compounds from AC are mainly classified into benzenoids, diterpenoids, triterpenoids, and polysaccharides [3]. Among them, ergostane-type triterpenoids are particular to the fruiting body of AC and have been proven to exhibit anti-inflammatory and cytotoxic activities [4–10]. The commercial products of AC fruiting body are both popular and precious in Taiwan; however, the abundances and distributions of active components in AC are significantly different from distinct culturing methods, manufacture, or extraction technique.

Typically, natural products can be analyzed using various detection techniques including ultraviolet or photodiode array, fluorescence, evaporative light scattering, and mass spectrometric detection [11–15]. High-performance liquid chromatography (HPLC) with ultraviolet (UV) and photodiode array (PDA) detection methods has become a routine practice in some laboratories to quantify benzenoids and triterpenoids derivatives of fruiting body, mycelia, extract, and commercial preparation of AC [16–19]. These methods have played important roles in the quality control of AC and its commercial products. However, due to the limitations of UV and PDA detectors in discriminating target analytes from interfering impurities, these methods require time-consuming sample pretreatment and long HPLC running times [20]. In addition, HPLC with UV detection only can provide information about retention time (t_R) of analyte, which may lead to a misleading description or false-positive quantification result when a non-target compound appears or coelutes at the same t_R as target analyte. To avoid these restrictions, a more sensitive liquid chromatography tandem mass spectrometry (LC–MS/MS), which can provide more overall information including retention time, molecular weight, and collision fragments of analyte, was performed in this study. Due to its high performance and efficiency, LC–MS/MS has been becoming a powerful analytical technique for qualitative and quantitative analysis of natural products [21–25]. Although few studies have demonstrated the chemical profile qualitation of AC by HPLC–electrospray ionization (ESI)–MS [26, 27], no validated multiple reaction monitoring (MRM) quantitative method has been reported. Thus, for

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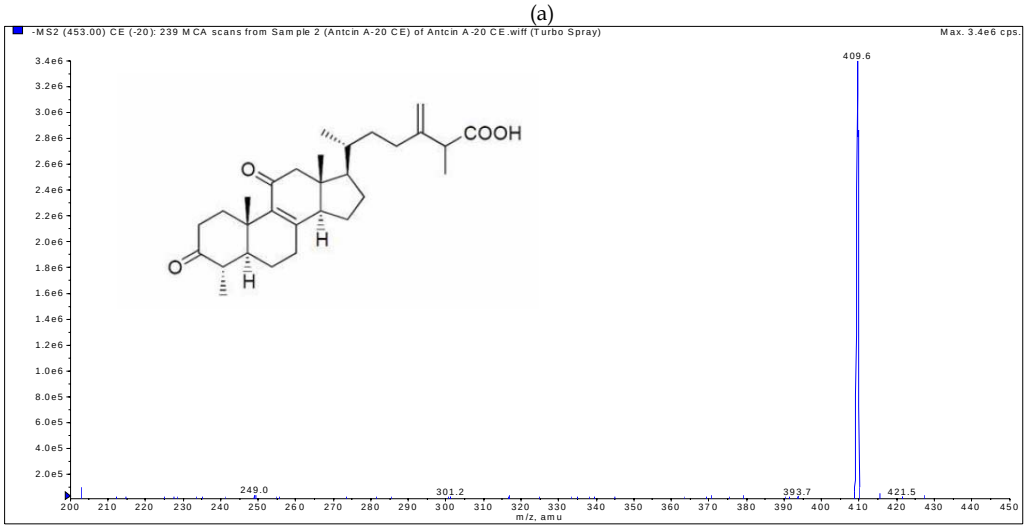

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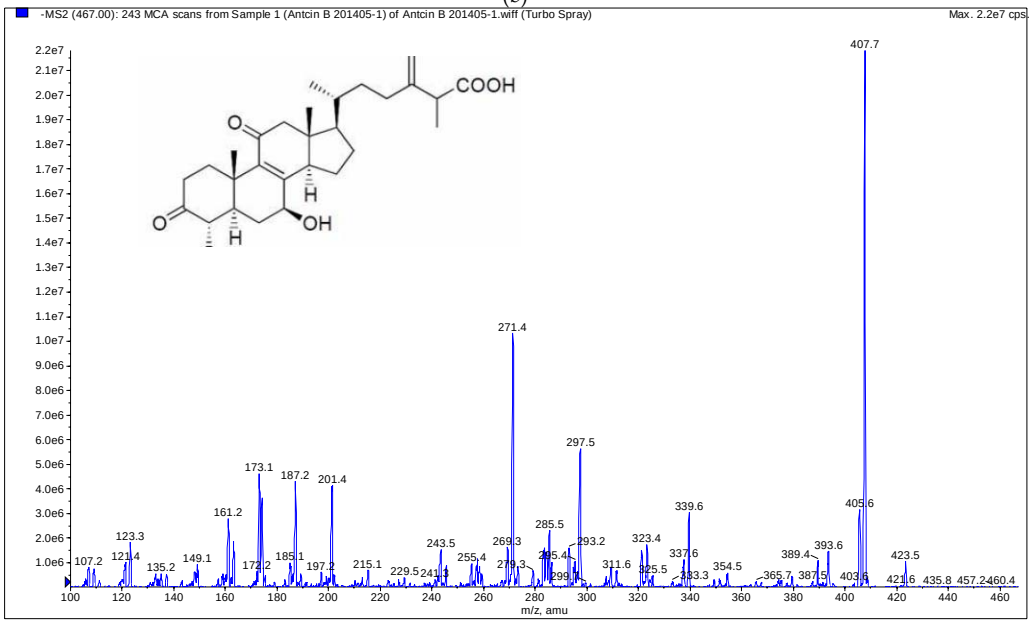
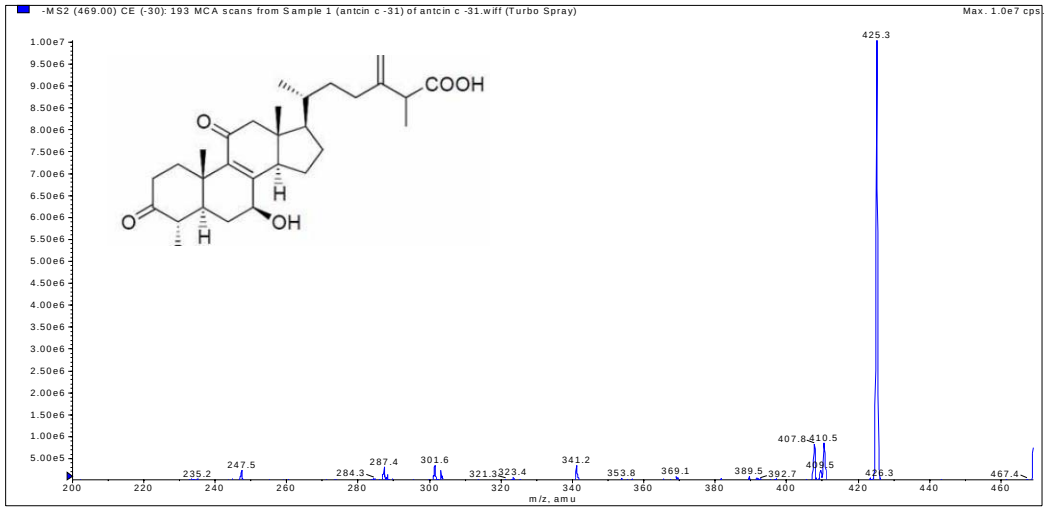


Fig. 1. (a) ESI (−) product ion spectrum of antcin A (*m*/*z* 453), (b) ESI (−) product ion spectrum of antcin B (*m*/*z* 467), (c) ESI (−) product ion spectrum of antcin C (*m*/*z* 469), (d) ESI (−) product ion spectrum of antcin H (*m*/*z* 485), (e) ESI (−) product ion spectrum of antcin K (*m*/*z* 487), (f) ESI (−) product ion spectrum of dehydroeburicoic acid (*m*/*z* 467), and (g) ESI (+) product ion spectrum of 4,7-dimethoxy-5-methyl-1,3-benzodioxole (*m*/*z* 197)

(c)



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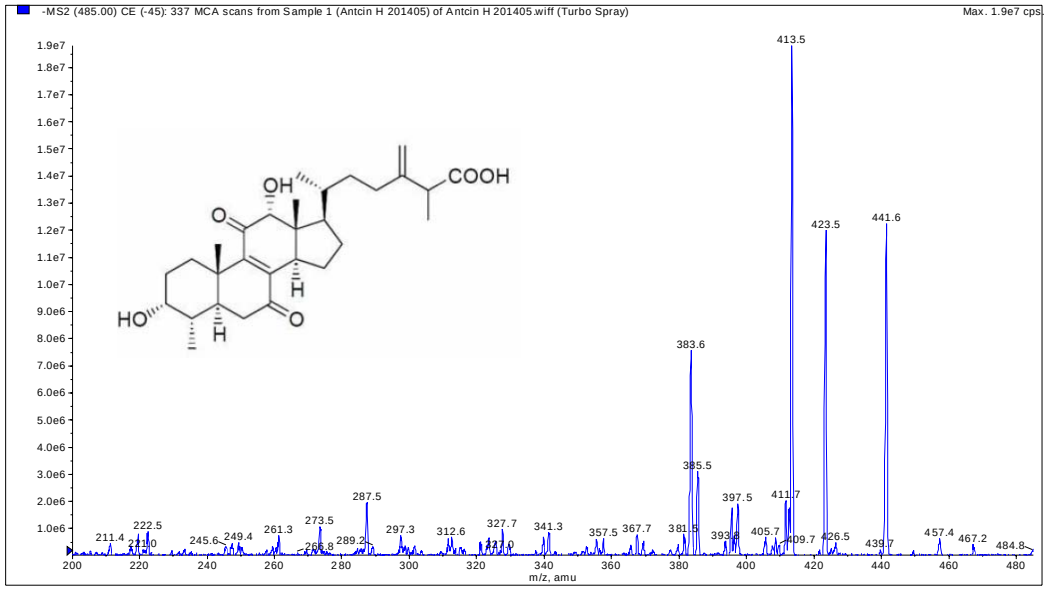
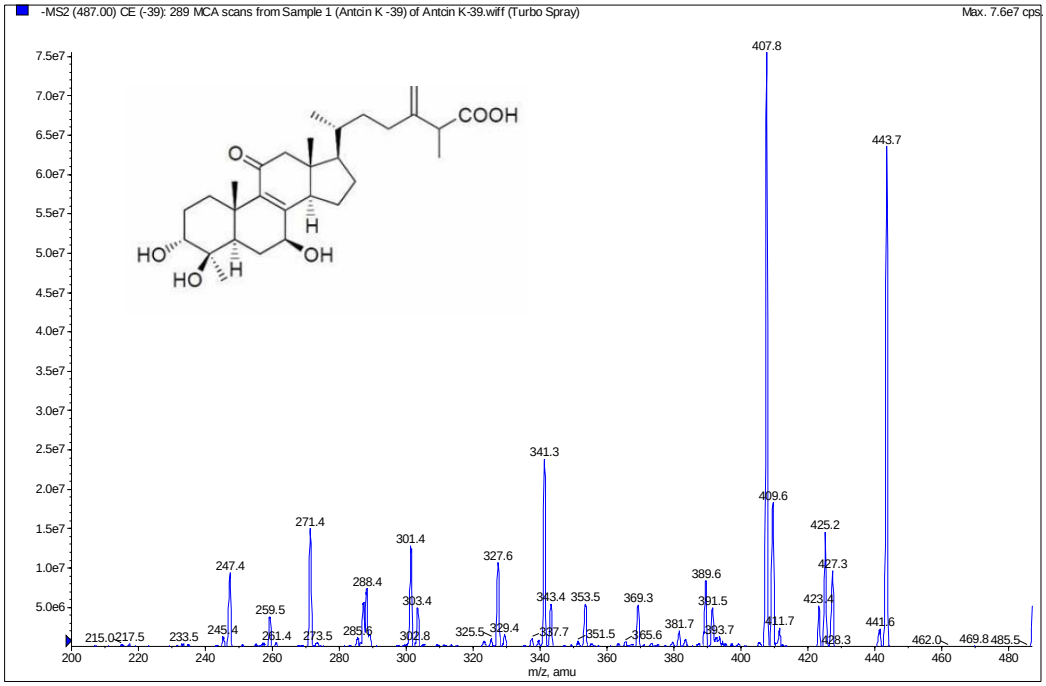


Fig. 1. (continued)

(e)



(f)

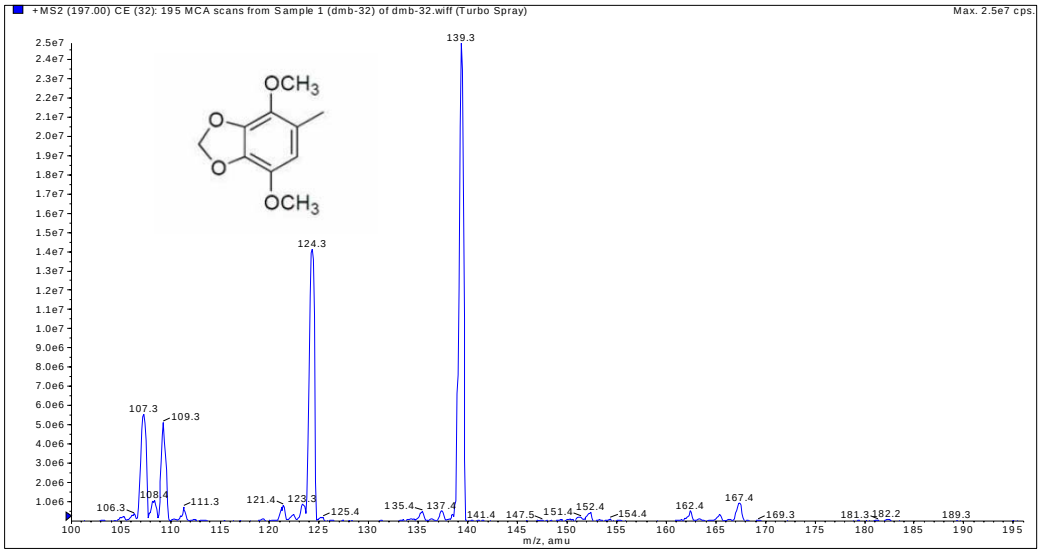


Fig. 1. (continued)

the purpose of developing a better quality control of AC derived products, an efficient and validated method for the profiling of signature compounds in AC fruiting body is necessary. In this study, the MRM method was established to identify and quantify the major constituents of AC for the first time.

Typically, the mass spectrometry data can be acquired from full scan, selected ion monitoring, product ion scan, precursor ion scan, and selected reaction monitoring or multiple reactions monitoring [28]. Among these, multiple reactions monitoring modes, which hold fixed transmissions of precursor ion and product ion of analyte, can evidently reduce the interferences caused by matrix or nontarget compounds with similar mass- to-charge ratio and therefore provide an accurate and sensitive quantitative analysis. In this study, we presented a validated MRM method for the rapid determination of six major triterpenoid compounds, antcins A, B, C, H, and K, and dehydroeburicoic acid, and one aromatic compound, 4,7-dimethoxy-5-methyl-1,3-benzodioxole *(Fig. 1.)* in the fruiting body of AC and its commercial product. Its performances in terms of precision, stability, repeatability, and accuracy were demonstrated in this study.

Experimental

General Experimental Procedures

Nuclear magnetic resonance (NMR) spectra were recorded on a Varian Mercury plus 400 NMR spectrometer using $CDCl_3$ or Pyridine-d5 as solvent, and tetramethylsilane (TMS) or the residual solvent signal was used as internal shift reference. Thin-layer chromatography (TLC) was carried out on silica gel 60 F_{254} plates (Merck). For column chromatography, silica gel (230–400 mesh ASTM, Merck) was used. HPLC was performed on a Hitachi L-7000 chromatograph with a Lichrosorb Si gel 60 (5 μm) column (250 × 10 mm).

Chemicals and Materials

The fruiting body and capsule of AC were purchased from Chinese medicinal herbs store of Taipei. HPLC-grade acetonitrile and methanol were purchased from Merck. All other reagents were of analytical grade. Double distilled water was used throughout the study.

Extraction, isolation, and structure determination of reference standards

The oven-dried fruiting body of AC (100 g) was extracted with ethanol under reflux for 1 h for three times. The combined extract was concentrated under reduced pressure, and the residue (16.2 g) was subjected to silica gel chromatography (5 × 45 cm) using a gradient mixture of *n*-hexane and EtOAc as eluent. Eluates were monitored by TLC using CH_2Cl_2–EtOAc, 9:1, and CH_2Cl_2–MeOH, 5:1, as developing solvent and combined into seven fractions according to the result of TLC analysis. Fraction 2 (*n*-hexane–EtOAc, 14:1; 1.2 g) was further purified through a silica gel column (2 × 45 cm), eluted with *n*-hexane–CH_2Cl_2 (10:1 to 0:1) to give six fractions, 2A–2F. Fr. 2B (321 mg) was subjected to semipreparative HPLC eluted with *n*-hexane–CH_2Cl_2–EtOAc (40:40:1) to obtain 4,7-dimethoxy-5-methyl-1,3-benzodioxole (16.2 mg). Fraction 4 (*n*-hexane–EtOAc, 7:3; 2.2 g) was rechromatographed on silica gel column (2 × 45 cm), eluted with CH_2Cl_2–EtOAc (40:1 to 1:1) to give seven fractions, 4A–4G. Fr. 4B (895 mg) was subjected to semipreparative HPLC eluted with CH_2Cl_2–acetone (35:1) to yield dehydroeburicoic acid (2.8 mg). Fr. 4D (76 mg) was subjected to semipreparative HPLC eluted with CH_2Cl_2–acetone (30:1) to yield antcin A (8.0 mg). Fraction 5 (*n*-hexane–EtOAc, 6:4; 3.1 g) was further chromatographed on a silica gel column (2 × 45 cm), eluted with CH_2Cl_2–EtOAc (10:1 to 0:1) to give eight fractions, 5A–5G. Fr. 5D (955 mg) was subjected to semipreparative HPLC eluted with CH_2Cl_2–acetone (25:1) to yield antcin B (26.3 mg). Fraction 6 (*n*-hexane–EtOAc, 4:6; 2.6 g) was further chromatographed on a silica gel column (2 × 45 cm), eluted with CH_2Cl_2–methanol (50:1) to give six fractions, 6A–6F. Fr. 6B (95 mg) was subjected to semipreparative HPLC eluted with CH_2Cl_2–EtOAc (10:1) to yield antcin C (3.2 mg). Fr. 6D (425 mg) was subjected to semipreparative HPLC eluted with CH_2Cl_2–acetone (8:1) to yield antcin H (31 mg). Fraction 7 (*n*-hexane–EtOAc, 1:9; 3.3 g) was further purified through a silica gel column (2 × 45 cm), eluted with CH_2Cl_2–MeOH (15:1 to 0:1) to yield antcin K (41.3 mg). Their structures of reference standards, antcins A [1], B [1], C [1], H [1], and K [7], 4,7-dimethoxy-5-methyl-1,3-benzodioxole [29], and dehydroeburicoic acid [30] were identified by comparing their ^1H- and ^{13}C-NMR values with those described in the literature *(Fig. 1)*. The isolated reference standards are confirmed with more than 98% purity on the basis of UV, MS, NMR, and HPLC analysis.

Preparation of standard solutions

Each standard stock solution was prepared by dissolving accurately weighed amount of compound in methanol. The standard mixture was diluted to a series of appropriate concentrations for the construction of calibration curves. The concentration range (in μg mL^{-1}) for each calibration curve is given as follows: antcin A, 10–200; antcin B, 10–500; antcin C, 10–200; antcin H, 10–500; antcin K, 10–500; dehydroeburicoic acid, 10–200; and 4,7-dimethoxy-5-methyl-1,3-benzodioxole, 10–100. All solutions were stored at 4 °C in a refrigerator until use and filtered through a 0.22-μm micropore membrane before injection.

Sample preparation

The fruiting body and capsule of AC were pulverized and screened through a 100-mesh sieve. The fine powders of two samples were accurately weighed (0.5 g) and placed in 10-mL volumetric flasks. They were extracted with 10 mL of methanol in an ultrasonic bath for 30 min at room temperature. Additional methanol was added to make up to the volume. The supernatant was filtered through a 0.22-μm micropore membrane prior to analysis.

LC/MS/MS analytical conditions

Quantitative LC–MS/MS analysis was performed using an Agilent 1100 HPLC system (Agilent Technologies) coupled to an API 4000 triple quadrupole tandem mass spectrometer (Applied Biosystem, Foster City, CA, USA). Chromatographic separation was performed on a Poroshell 120 C18 column (100 × 3.0 mm I.D., 2.7 μm; Agilent, CA, USA). The mobile phase consisted of 0.2% formic acid aqueous solution (solution A) and acetonitrile (solution B), and a gradient elution program was set as follows: solution A, 70–50% (0–7.5 min), 50–40% (7.5–10.8 min), 40–0% (10.8–19 min), 0–40% (19–21 min), 40–50% (21–25 min), and 50–70% (25–30 min). The column temperature was fixed at 22 °C, the flow rate was set 0.5 mL min^{-1}, and injection volume was 10 μL. The electrospray negative mode was selected as an ion source for antcins A, B, C, H, and K and dehydroeburicoic acid detection. The positive electrospray mode was selected as an ion source for 4,7-dimethoxy-5-methyl-1,3-benzodioxole detection. The quantification was performed in multiple reactions monitoring (MRM). The optimized ESI

source parameters were as follows: ion spray voltage, −4500 V for negative mode and 4500 V for positive mode; nitrogen nebulizer gas pressure, 12 psi; nitrogen curtain gas pressure, 10 psi; heater temperature, 450 °C; and collisionally activated dissociation (CAD) gas, 6 psi. The precursor-to-product ion transitions were *m/z* 453/409, *m/z* 467/408, *m/z* 469/425, *m/z* 485/413, *m/z* 487/407, *m/z* 467/337, and *m/z* 197/139 for antcin A, antcin B, antcin C, antcin H, antcin K, dehydroeburicoic acid and 4,7-dimethoxy- 5-methyl-1,3-benzodioxole, respectively. Their optimized declustering potentials (DP), collision energies (CE), and collision cell exit potential (CXP) were listed in *Table I*. All data acquisition and processing were performed using Analyst 1.4.1 software (AB SCIEX, Concord, ON, Canada).

Table I. MRM transitions, declustering potential (DP), collision energy (CE), and collision cell exit potential (CXP) of seven signature compounds

Compound	MRM transition (*m/z*)	DP (V)	CE (eV)	CXP (V)
Antcin K	487 > 407	−132	−37	−8
4,7-Dimethoxy-5-methyl-1,3-benzodioxole	197 > 139	25	21	10
Antcin H	485 > 413	−128	−37	−10
Antcin C	469 > 425	−117	−30	−15
Antcin B	467 > 408	−124	−40	−10
Antcin A	453 > 409	−117	−29	−12
Dehydroeburicoic acid	467 > 337	−194	−48	−8

Calibration curve, limits of detection, and limits of quantification

Solutions containing seven standards at five different concentrations were injected in triplicate. The calibration curve of each analyte was constructed by plotting the peak area versus the corresponding concentration. The mixed standards solution was further diluted to a certain concentration to explore the limits of detection (LOD) and quantification (LOQ). The LOD and LOQ were determined at a signal-to-noise (S/N) ratio of 3 and 10, respectively.

Precision, stability, repeatability, and accuracy

The intra- and inter-day precisions were determined by continuously injecting the sample solution for three replicates on the same day and by measuring it once a day for three consecutive days, respectively. The recovery test for reflecting accuracy was done by the standard addition approach. The recovery yield was figured out according to the following formula: recovery yield (%) = [(amount detected − original amount)/amount spiked] × 100%, and RSD (%) = (SD/mean) × 100%. The repeatability was estimated on the ground of relative standard deviation (RSD).

Determination of content

The peak area of each component in the fruiting body and capsule of AC was acquired from its MRM chromatogram, and the abundance of each compound was calculated from its corresponding calibration curve. Experiments were done in triplicate, and the resulting data were expressed as the mean ± SE and the unit was represented as mg g^{-1}.

Results and Discussion

Optimization of LC–MS/MS Conditions

With the ESI source of the mass spectrometer, antcins A, B, C, H, and K, and dehydroeburicoic acid showed better sensitivity in the negative ion mode, but 4,7-dimethoxy-5-methyl-1,3-benzodioxole exhibited a higher sensitivity in the positive ion mode *(Fig. 1)*. Given this discrepancy, a positive–negative conversion multiple reaction monitor was used to determine the content of the above seven compounds. The ion spray voltage was limited to 4500 V to reduce in-source dissociation, and the source temperature increased to 450 °C to improve the response of these compounds. Other parameters were adjusted suitably to optimize ionization. Product ion spectra of seven compounds are shown in *Fig. 1*. The most characteristic precursor-to-product ion transitions were finally chosen to be *m/z* 453/409, *m/z* 467/408, *m/z* 469/425, *m/z* 485/413, *m/z* 487/407, *m/z* 467/337, and *m/z* 197/139 for antcin A, antcin B, antcin C, antcin H, antcin K, dehydroeburicoic acid, and 4,7-dimethoxy-5-methyl-1,3-benzodioxole, respectively. The precursor-to-product ion pair, declustering potential (DP), collision energy (CE), and collision cell exit potential (CXP) for each analyte were also optimized *(Table I)*.

Method Validation

The MRM profiles are shown in *Fig. 2*. The method showed excellent linear response in the selected concentration range *(Table II)*. The limits of detection (LOD) of seven compounds were 0.12, 0.11, 0.20, 0.15, 0.35, 0.70, and 0.65 μg mL^{-1} for antcin A, antcin B, antcin C, antcin H, antcin K, dehydroeburicoic acid, and 4,7-dimethoxy-5-methyl-1,3-benzodioxole based on a signal-to-noise ratio of 3:1, respectively. The precision of the method was tested by measuring both intra-day and inter-day reproducibility from analysis of standard solutions at three different levels. The intra-day reproducibility of the assay was determined using standard solution within the same day. The inter-day variability of the assay was determined by three injections on three different days. The results in *Table III* showed that RSD values in peak areas were all below 3% at three levels for seven components. Recovery experiments were performed at three concentration levels by adding an appropriate amount of standards solution to the blank samples. Recovery yields for spiked blank samples were in the range of 87%–96% with RSD < 3% for all analytes.

Table II. Retention time, MRM transition, calibration curve data, limit of detection (LOD), and limit of quantification in MRM mode for LC–MS/MS analysis

Compound	Retention time (t_R)	MRM transition (m/z)	Regression equation	R^2	Range (μg mL^{-1})	LOD (μg mL^{-1})	LOQ (μg mL^{-1})
Antcin K	6.7	487 > 407	$y = 595.3x + 3678.7$	0.9989	10–500	0.35	1.05
4,7-Dimethoxy-5-methyl-1,3-benzodioxole	9.2	197 > 139	$y = 206.0x - 717.5$	0.9990	10–200	0.65	1.95
Antcin H	12.5	485 > 413	$y = 2367.9x + 18045.0$	0.9995	10–500	0.15	0.45
Antcin C	13.1	469 > 425	$y = 3104.6x - 846.6$	0.9978	10–200	0.20	0.60
Antcin B	14.6	467 > 408	$y = 1499.1x + 7307.1$	0.9990	10–500	0.11	0.33
Antcin A	15.9	453 > 409	$y = 1300.0x + 4556.2$	0.9992	10–200	0.12	0.36
Dehydroeburicoic acid	19.0	467 > 337	$y = 124.9x + 254.0$	0.9991	10–200	0.70	2.10

Table III. Precisions and recovery yields of intra-day and inter-day experiments of the seven compounds

Compound	Precision (%) RSD		Recovery (%)	
	Intra-day (n = 3)	Inter-day (n = 3)	Average	RSD, %
Antcin K	1.51	2.53	87.55	2.55
4,7-Dimethoxy-5-methyl-1,3-benzodioxole	1.32	2.41	95.41	2.69
Antcin H	1.97	2.12	89.54	2.97
Antcin C	1.97	2.12	91.30	2.47
Antcin B	1.72	1.99	93.41	2.02
Antcin A	1.43	1.79	90.24	2.43
Dehydroeburicoic acid	1.87	2.07	89.44	2.91

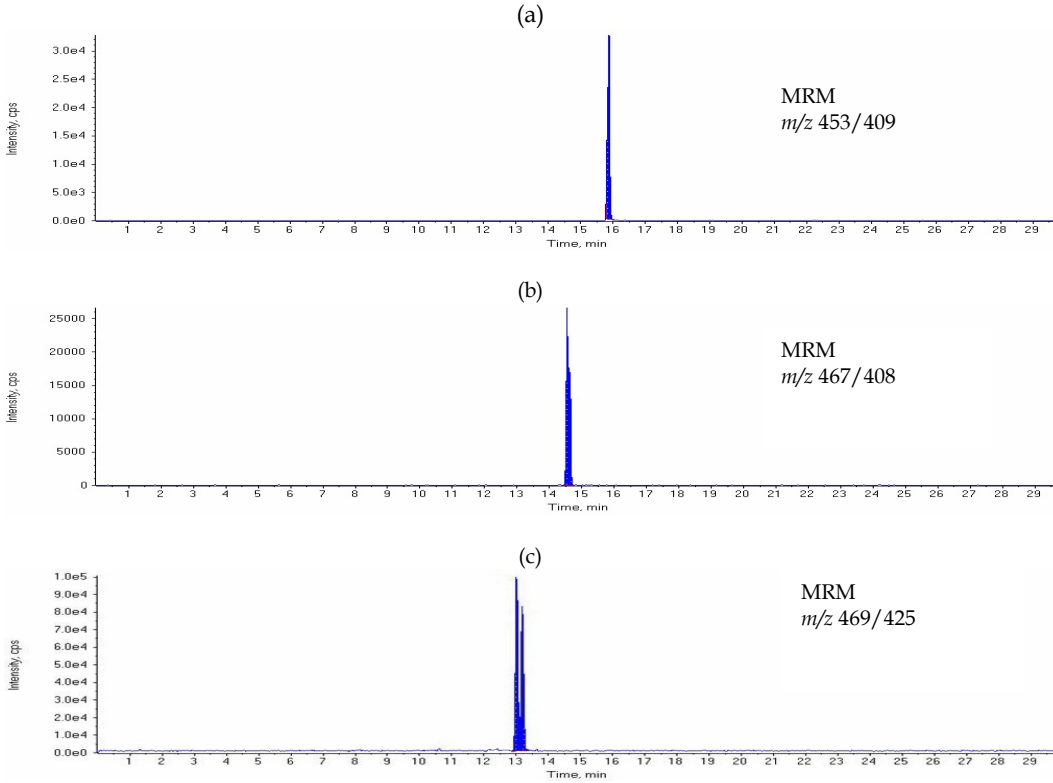


Fig. 2. MRM chromatograms of (a) antcin A, (b) antcin B, (c) antcin C, (d) antcin H, (e) antcin K, (f) dehydroeburicoic acid, and (g) 4,7-dimethoxy-5-methyl-1,3-benzodioxole

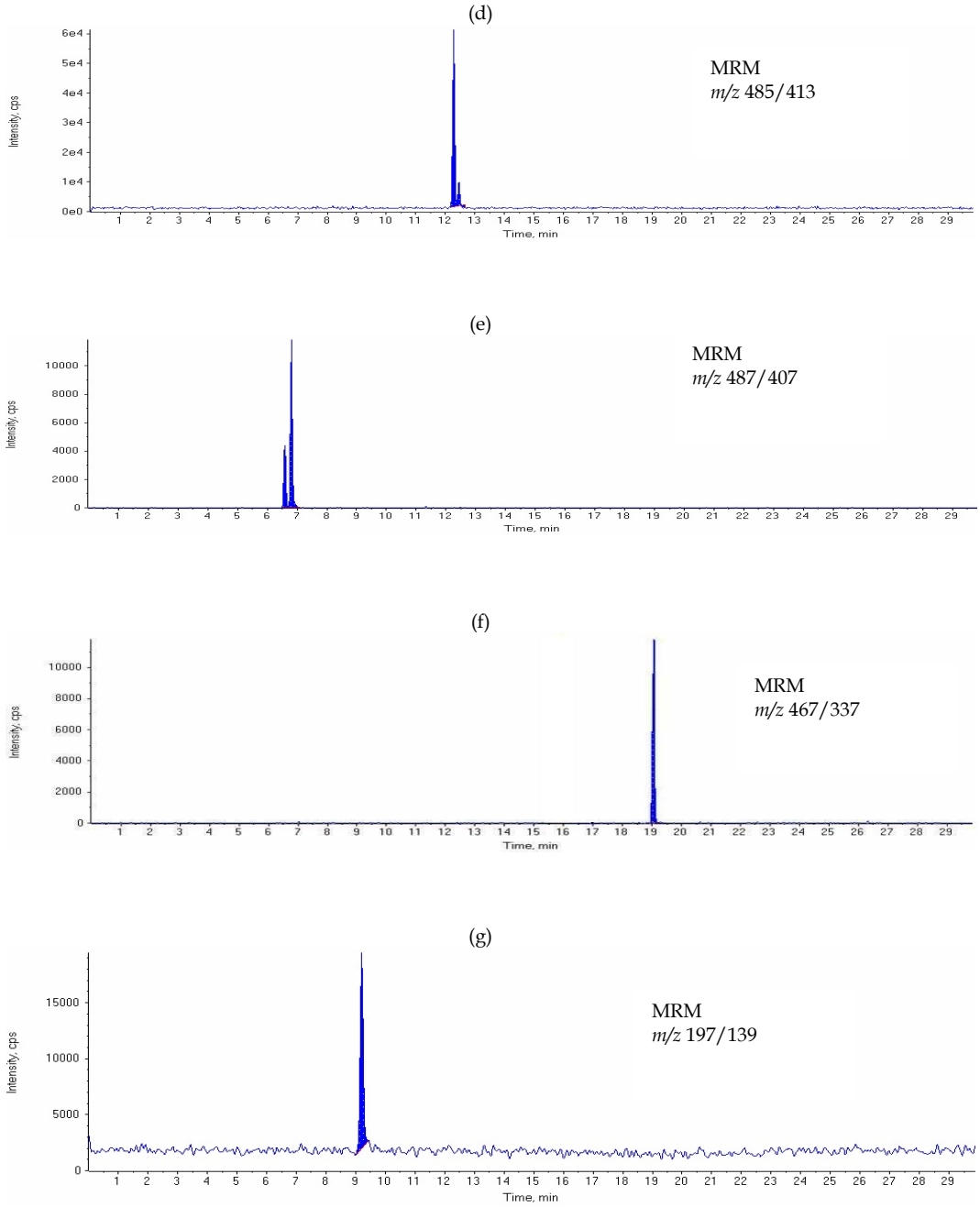


Fig. 2. (continued)

Sample Analysis

The described HPLC–MS/MS method was subsequently applied to analysis and quality evaluation of fruiting body and capsule of AC through simultaneous determination of seven marker compounds. The quantitative analytical results *(Table IV)* indicated their contents distributed in these samples. In the extract of AC fruiting body, antcin H with the highest abundance (4.08 mg g^{-1}) and 4,7-dimethoxy-5-methyl-1,3-benzodioxole (0.35 mg g^{-1}) with the lowest content among seven signature compounds were simultaneously obtained. In the capsule of AC body, the abundances of seven signature components were lower than those found in the AC fruiting body. The contents of antcins A, B, C, H, and K and dehydroeburicoic in the capsule of AC fruiting body were in the range of 0.23–1.51 mg g^{-1}, and the abundance of 4,7-dimethoxy-5-methyl-1,3-benzodioxole was found at 0.17 mg g^{-1}. The quality of botanical medicine preparation varied with pretreatment, manufacturing process, and dosage form. Meanwhile, the content of bioactive markers in the commercial products may be also affected by different planting regions of material, cultivated time, harvest season, geographical climate, and environment parameters. To ensure the stability, safety and efficacy for clinical use, guidelines, and quality control for AC, preparations should be standardized.

Table IV. Contents of seven signature compounds in fruiting body and capsule of *Antrodia cinnamomea*

Compound	[a]Dried fruiting body (mg g^{-1})	[a]Capsule (mg g^{-1})
Antcin K	3.91 ± 0.23	1.45 ± 0.08
4,7-Dimethoxy-5-methyl-1,3-benzodioxole	0.35 ± 0.05	0.17 ± 0.07
Antcin H	4.08 ± 0.37	1.51 ± 0.05
Antcin C	1.27 ± 0.19	0.57 ± 0.06
Antcin B	3.80 ± 0.75	1.01 ± 0.34
Antcin A	0.89 ± 0.24	0.23 ± 0.01
Dehydroeburicoic acid	1.10 ± 0.12	0.40 ± 0.05

[a]Data represented as mean (mg g^{-1}) ± SD, n = 5.

Conclusion

Previously, Lin et al. had reported the quantitative determination method of AC by HPLC with ultraviolet detection, requiring a long HPLC running time [17]. HPLC with mass spectrometry detection to evaluate the quality of AC mycelium was also conducted by Zhao and Leung, but the metabolite composition of the fruiting bodies of AC has not been evaluated [31]. Du et al. had determined the triterpenoids of AC by LC–MS; however, their method was not validated [26]. In this study, we developed a validated HPLC–MS/MS method for the rapid and accurate quantification of seven signature compounds in AC. The overall time required for chromatographic separation (including sample injection and column equilibration) is within 30 min. The applicability and reliability of this analytical approach was confirmed by the method validation and the successful analysis of real samples of extract and preparation of AC. In conclusion, this validated HPLC–MS/MS method offers promising applications for the accurate and rapid quantification of signature components in various samples, which will benefit the development of AC fruiting body derived products.

Acknowledgments

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References

[1] I.H. Cherng, H.C. Chang, M.C. Cheng, and Y. Wang, J. Nat. Prod., **58**, 365 (1995)
[2] T.T. Chang and W.N. Chou, Bot. Bull. Acad. Sin., **45**, 347–355 (2004)
[3] Z.H. Ao, Z.H. Su, Z. Lu, H.Y. Xu, X.M. Zhang, and W.F. Dou, J. Ethnopharmacol., **121**, 194 (2009)
[4] I.H. Cherng, D.P. Wu, and H.C. Chiang, Phytochmeistry, **41**, 263 (1996)
[5] Y.C. Shen, C.F. Chen, Y.H. Wang, T.T. Chang, and C.J. Chou, Pharm. J., **55**, 313 (2003)
[6] C.C. Shen, Y.H. Wang, T.T. Chang, L.C. Lin, M.J. Don, Y.C. Hou, K.T. Liou, S. Chang, W.Y. Wang, H.C. Ko, and Y.C. Shen, Planta. Med., **73**, 1208 (2007)
[7] C.H. Chen, S.W. Yang, and Y.C. Shem, J. Nat. Prod., **58**,1655 (1995)
[8] J.J. Chen, W.J. Lin, C.H. Liao, and P.C. Shieh, J. Nat. Prod., **70**, 989 (2007)
[9] K.B. Male, Y.K. Rao, and Y.M. Tzeng, Chem. Res. Toxicol., **21**, 2127 (2008)

[10] C.T. Yeh, Y.K. Rao, C.J. Yao, C.F. Yeh, C.H. Li, S.E. Chuang, J.H. Luong, G.M. Lai, and Y.M. Tzeng, Cancer. Lett., **285**, 73 (2009)
[11] D. Steinmann and M. Ganzera, J. Pharm. Biomed. Anal., **55**, 744 (2010)
[12] C. Ho, C. Lam, M. Chan, R. Cheung, L. Law, L. Liu, K. Ng, M. Suen, and H. Tai, Clin. Biochem. Rev., **24**, 3 (2003)
[13] J. Xing, C.F. Xie, and H.X. Lou, J. Pharm. Biomed. Anal., **44**, 368 (2007)
[14] M. Yang, J. Sun, Z. Lu, G. Chen, S. Guan, X. Liu, B. Jiang, M. Ye, and Da. Guo, J. Chromatogr. A, **1216**, 2045 (2009)
[15] J.L. Zhou, L.W. Qi, and P. Li, J. Chromatogr. A, **1216**, 7582 (2009)
[16] C.Y. Chang, M.Y. Lue, and T.M. Pan, J. Food. Drug. Anal., **13**, 338 (2005)
[17] T.Y. Lin, C.Y. Chen, S.Y. Chien,, W.W. Hsiao, F.H. Chu, W.H. Li, C.C. Lin, J.F. Shaw, and S.Y. Wang, J. Agric. Food. Chem., **59**, 7626 (2011)
[18] S.H. Tu, C.H. Wu, J.C. Chen, C.S. Huang, H.W. Chang, C.H. Chang, H.M. Lien, and Y.S. Ho, J. Agric. Food. Chem., **60**, 3612 (2012)
[19] H.C. Wang, F.H. Chu, S.C. Chien, J.W. Liao, H.W. Hsieh, W.H. Li, C.C. Lin, J.F. Shaw, Y.H. Kuo, and S.Y. Wang, J. Agric. Food. Chem., **61**, 8556 (2013)
[20] C.H. Huang, Y.Y. Chang, C.W. Liu, W.Y. Kang, Y.L. Lin, H.C. Chang, and Y.C. Chen, J. Agric. Food. Chem., **58**, 3859 (2010)
[21] U. Justesen, J. Chromatogr. A, **902**, 369 (2000)
[22] H.J. Zhang, P. Shen, and Y.Y. Cheng, J. Pharm. Biomed. Anal., **34**, 705 (2004)
[23] Y.J. Hsieh, L.C. Lin, and T.H. Tsai, J. Chromatogr. A, **1083**, 141 (2005)
[24] D.P. Singh, R. Govindarajan, and A.K.S. Rawat, Phytochem. Anal., **19**, 164 (2008)
[25] A. Niranjan, J. Barthwal, A. Lehri, D.P. Singh, R. Govindrajan, A.K.S. Rawat, and D.V. Amla, Acta Chromatogr., **21**, 105 (2009)
[26] Y.C. Du, T.Y. Wu, F.R. Chang, W.Y. Lin, Y.M. Hsu, F.T. Cheng, C.Y. Lu, M.H. Yen, Y.T. Tsui, H.L. Chen, M.F. Hou, M.C. Lu, and Y.C. Wu, J. Pharm. Biomed. Anal., **58**, 182 (2012)
[27] Y. Liu, X. Di, X. Liu, W. Shen, and K.S. Leung, J. Pharm. Biomed. Anal., **53**, 781 (2010)
[28] X.G. He, J. Chromatogr. A, **880**, 203 (2000)
[29] C.C. Shen, Y.C. Kuo, R.L. Huang, L.C. Lin, M.J. Don, T.T. Chang, and C.J. Chou, J. Chin. Med., **14**, 247 (2003)
[30] H.C. Chiang, D.P. Wu, I.W. Cherng, and C.H. Ueng, Phytochemistry, **39**, 613 (1995)
[31] S.S. Zhao and S.Y. Leung. Chin. Med., **5**, 4 (2010)