Waters™

Applikationsbericht

Benefits of Mass Detection Using the ACQUITY QDa Mass Detector for Routine Botanical Authentication

Jinchuan Yang, Jimmy Yuk, Paul D. Rainville

Waters Corporation

Abstract

In this feasibility study, a method for botanical authentication was developed using the MS data collected by the ACQUITY QDa Mass Detector. The accuracy of this authentication method was evaluated in a blind test with four commercial black cohosh samples. The advantages of MS over using a evaporative light scattering detector (ELSD) are also highlighted.

Benefits

- Provides a simple and affordable MS detector for botanical authenticity testing.
- Generates highly selective and distinctive MS data suitable for complex samples.
- Streamlines the routine botanical authentication data processing.

Introduction

Botanical ingredients are widely used in dietary supplements, herbal medicines, cosmetics, and personal care products. Potential contamination or misidentification of plants has been a health concern due to the lack of standardization of production. The testing of botanical ingredients and processed products for authenticity is a challenging task due to their complex phytochemical constituents, the variation in chemical profiles from different locations, plant anatomy, age and harvest season, and the similar phytochemical profile of closely related species. Many analytical techniques are used in botanical authenticity testing.[1] Liquid chromatography (LC) coupled with mass spectrometry (MS) is one of the most effective tools.[2-7] However, LC-MS has not been widely employed in analytical labs for routine authenticity testing due to the relatively high cost of mass spectrometers and the high level of expertise required.

Waters ACQUITY QDa Mass Detector is an affordable and easy-to-use mass spectrometer designed for chromatographers. It offers a practical solution for bringing LC-MS to the routine analysis lab environment. In this application note, the feasibility of using mass detection for authenticity testing is demonstrated in a study for North American (NA) black cohosh (*Actaea racemosa*). In this feasibility study, a method for botanical authentication was developed using the MS data collected by the ACQUITY QDa Mass Detector. The accuracy of this authentication method was evaluated in a blind test with four commercial black cohosh samples. The advantages of MS over using a evaporative light scattering detector (ELSD) are also

highlighted.

Experimental

Sample preparation

Three authentic NA black cohosh, *Actaea racemosa* extracts (NA1-3), three Asian black cohosh, *Actaea cimicifuga* extracts (A1-3), and four commercial black cohosh samples (U1-4) were provided by a collaborator. These extracts were diluted with 70% methanol to about 5 mg/mL. Four standards: cimifugin, cimiracemoside C (cimigenol-3-alpha-L-arabinoside), 27-deoxyactein (23-epi-26-deoxyactein), and actein were purchased from ChromaDex (Irvine, CA). These standards were prepared in 70% methanol at about 5 µg/mL. The standard structures, CAS Registry numbers, and monoisotopic masses are shown in Figure 1. Homemade black cohosh samples, M-5 and M-10, were prepared by mixing NA black cohosh sample (NA1) and Asian black cohosh (A1) at 95:5 and 90:10 mass ratios, respectively. Sample solutions were filtered by 0.2 micron PTFE membrane prior to the analysis.

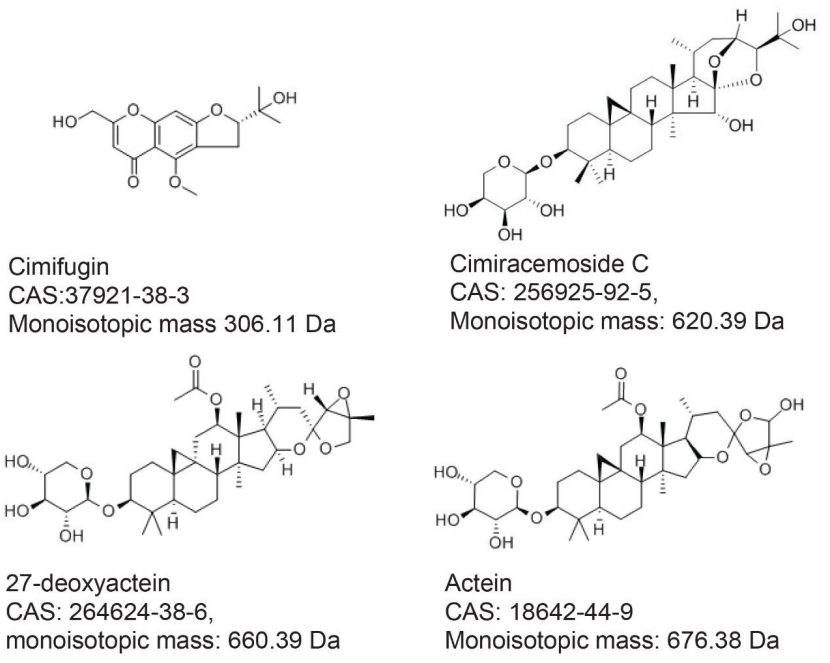

Figure 1. Structures, CAS Registry Numbers, and the monoisotopic masses of standards.

UPLC conditions

UPLC system:	ACQUITY UPLC H-Class
Column:	ACQUITY BEH C_{18}, 1.7 µm, 2.1 × 100 mm, (p/n 186002352)
Column temp.:	50 °C
Eluent A:	De-ionized water with 0.1% formic acid
Eluent B:	Acetonitrile/methanol (v/v 7/3) with 0.1% formic acid
Injection volume:	10 µL
Run time:	9 min
Reconditioning:	2.5 min

Time (min)	Flow rate (mL/min)	%A	%B	Curve
Initial	0.40	80	20	Initial
0.5	0.40	80	20	6
4.0	0.40	35	65	6
6.0	0.40	0	100	6
9.0	0.40	0	100	6
9.1	0.40	80	20	6

Table 1. UPLC elution gradient.

MS conditions

MS system:	ACQUITY QDa (with Diverter

	Valve)
Software:	Empower 3
Detection:	ESI+, MS scan
Scan:	200 to 1000 Da
Capillary voltage:	1.5 kV
Cone voltage:	10 V
Probe temp.:	300 °C
Sampling rate:	5 Hz
Diverter valve events:	Switch on/flow to QDa at 0.8 min; switch off/flow to waste at 9 min

ELSD conditions

Detector:	2424 ELS
Gain:	250
Pressure:	45.0 psi
Drift tube temp.:	55 °C
Nebulizer:	Heat at 50% power level
Data rate:	10 pps

Filter time constant:	Normal

Results and Discussion

UPLC-MS Method Optimization

The ACQUITY QDa Mass Detector's default setting is suitable for many applications. For this study, in order to get the maximum molecular ion intensity, the MS instrument parameters, such as the probe temperature, capillary voltage, and cone voltage were optimized. Figure 2 shows the effects of the cone voltage on the cimiracemoside C mass spectrum. The MS parameters that generated the maximum molecular ion intensity were selected and used, as described in the Experimental section. UPLC conditions[6] were used in this study with minor optimization.

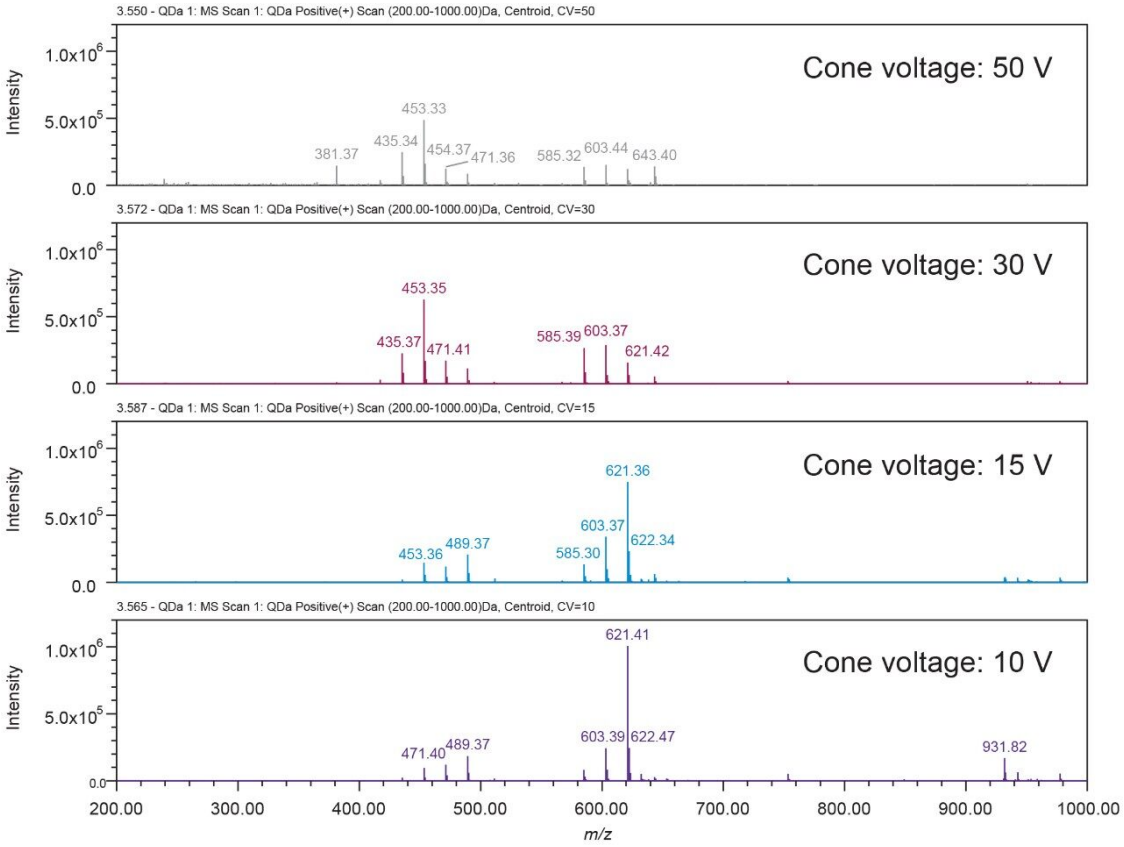

Figure 2. Effects of cone voltage on the cimiracemoside C mass spectrum. The cone voltages are shown in the spectra. Other MS conditions: probe temp. at 300 °C; capillary voltage at 1.5 kV.

Selection of Marker for the Authentic Black Cohosh

Authentic black cohosh, or NA black cohosh, is manufactured from the roots and rhizomes of *Actaea racemosa*. The potential contamination or misidentification of the black cohosh plant has been a health concern for many years due to a lack of standardization of production. Adverse events of hepatotoxicity associated with the use of black cohosh products have been reported, and it was suspected that unauthentic black cohosh might have contributed to some of the incidents.[8] Contamination or misidentification often occurs with the Chinese species of *Actaea* such as *A. heracleifolia, A. dahurica, and A. cimicifuga*, and NA *Actaea* species growing in the same area as NA black cohosh, such as *A. pachypoda, A. rubra, and A. podocarpa*. Many chemical constituents of black cohosh have been used as biomarkers for authenticity testing.[2-6] Among these markers, the most common ones, such as cimifugin, cimiracemoside C, actein, and 27-deoxyactein, were screened in this UPLC-MS study for suitable marker(s) to be used with the ACQUITY QDa Mass Detector (see Figure 3).

The molecular ions ($[M+H]^+$) of cimifugin, cimiracemoside C, and 27-deoxyactein, and the dehydration ions

($[M+H-H_2O]^+$) of actein were the dominant ions (base peak) in their respective mass spectra (data not shown). The mass-to-charge ratios (*m/z*) of these base peak ions were used to extract the ion chromatograms from the MS scan data, respectively. The resulting extracted ion chromatograms (XICs) are shown in Figure 3. After inspection of the XICs in Figure 3, the cimiracemoside C (*m/z* 621 Da, retention time 5.8 min in Figure 3B) was selected as the marker for NA black cohosh because it showed the most simple and distinctive chromatographic pattern in the NA black cohosh as compared to the pattern from the Asian black cohosh.

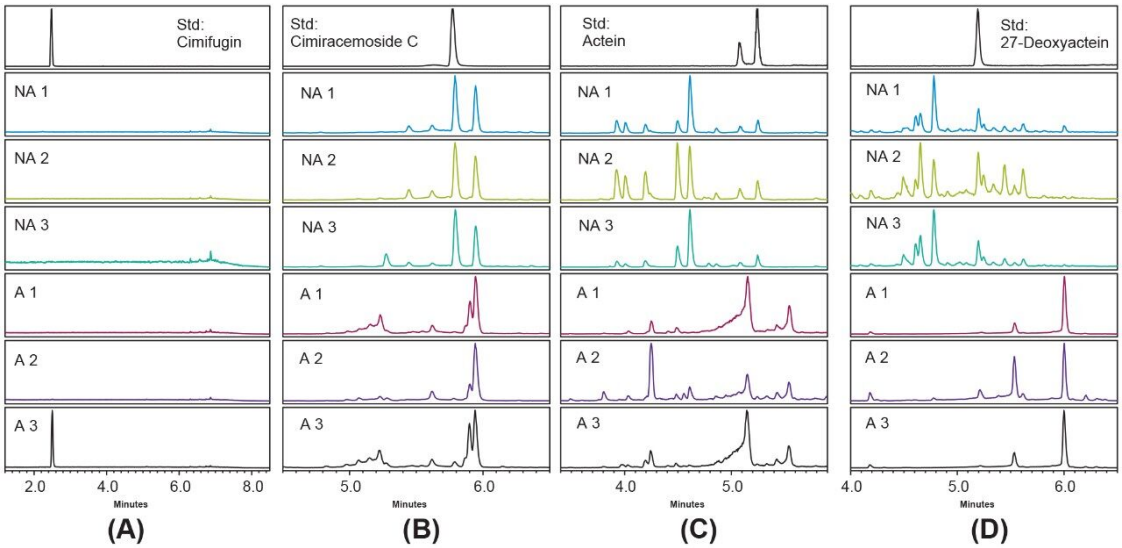

Figure 3. Extracted ion chromatograms of A. cimifugin (m/z 307 Da), B. cimiracemoside C (m/z 621 Da), C. actein (m/z 659 Da), and D. 27-deoxyactein (m/z 661 Da) in standards, NA black cohosh (NA 1-3), and Asian black cohosh (A 1-3) samples. For clarity, only the XIC sections of interest are shown.

Authenticity Data Processing Protocol

In order to make this authenticity method suitable for routine analysis, a new authenticity data processing protocol was designed. In this protocol the marker's distinctive chromatographic and mass spectral patterns, or fingerprints are used to evaluate sample authenticity (Figure 4). This authenticity data processing protocol was implemented in Empower 3 CDS Software using the existing functions. No special chemometric software program was used. Details of how this protocol was developed and implemented in Empower 3 Software are discussed in a second application note < https://www.waters.com/waters/library.htm?&cid=134864024&lid=134981491> .[9]

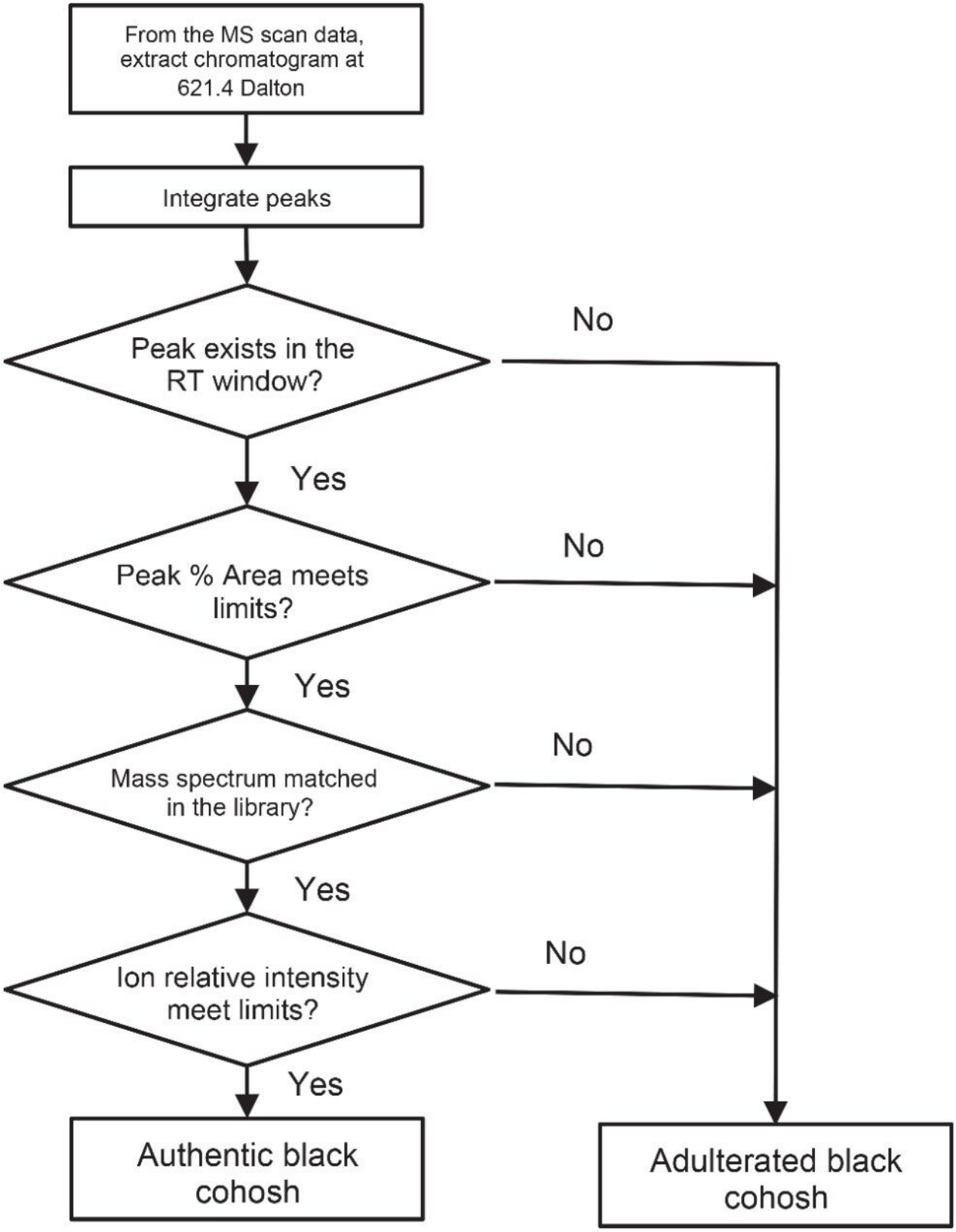

Figure 4. Authenticity data processing protocol for black cohosh using the chromatographic and the mass

spectral patterns of cimiracemoside C (marker).

To support this authenticity data processing protocol, a black cohosh MS library was created in Empower 3 Software. The cimiracemoside C mass spectra from the NA black cohosh samples were extracted (with baseline subtraction) from the MS scan data, and stored in the NA black cohosh MS library. Empower's MS Library Match function was used to check whether a sample's mass spectrum matched with any of the reference mass spectra in the library. An MS library can be custom built in Empower 3, exported and imported. Figure 5 shows an MS Library Match result. UV/Vis spectra can also be used for authenticity testing. However, UV/Vis spectra are usually less specific compared to mass spectra. In this case, the cimiracemoside C is UV transparent. So, it is not useful to use the UV/Vis spectral library for black cohosh authenticity testing.

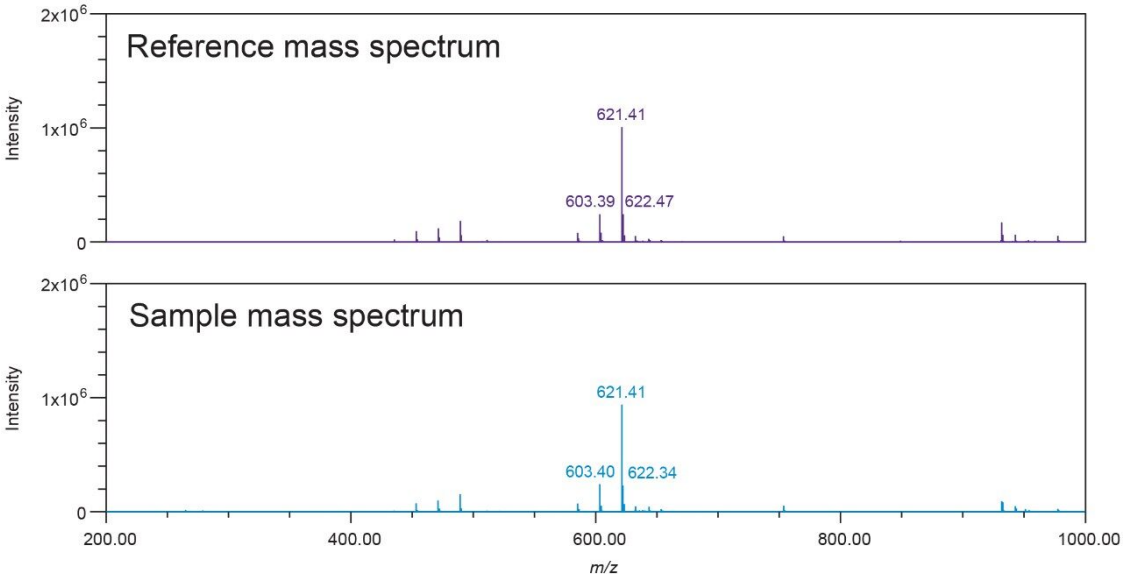

Figure 5. Empower 3 MS Library Match result. The mass spectrum of the marker (cimiracemoside C) peak in a sample (bottom) matches one of the reference mass spectra in the black cohosh MS library (top).

Analysis of Commercial and Homemade Black Cohosh Samples

Four commercial black cohosh samples (U1-4) and two homemade black cohosh samples (M-5 and M-10) were tested by this method. The commercial sample origins were revealed after the evaluation was completed. Table 2 is a summary of the authenticity test results for these samples. The authentic NA black

cohosh (U2 and U3) and the inauthentic black cohosh (U1 and U4) samples were correctly determined. The contaminated samples were also correctly detected, including the 5 wt% contaminated black cohosh sample (M-5). Details of how the homemade black cohosh samples were prepared can be found in the Experimental section.

Sample	Marker peak in RT window detected?	Marker peak relative area within threshold?	MS library match found?	Expected ion relative abundance within threshold?	Authentic black cohosh?	Sample origin
U 1	+	-	+	-	No	*Actaea Cimicifuga*
U 2	+	+	+	+	Yes	*Actaea racemosa*
U 3	+	+	+	+	Yes	*Actaea racemosa*
U 4	+	-	+	-	No	*Actaea dahurica*
M-5	+	+	+	-	No	Contamination at 5 wt%
M-10	+	+	+	-	No	Contamination at 10 wt%

Table 2. Black cohosh sample authenticity test results and sample origins.

Note: - negative answer; + positive answer.

Comparison of MS and ELSD for Authenticity Testing

Evaporative light scattering detector (ELSD) is commonly used for the black cohosh identification.[10] A comparison of the MS and the ELSD data for authenticity testing was made with an NA black cohosh sample (NA 1), an Asian black cohosh sample (A1), and four unknown black cohosh samples (U 1-U 4), as shown in Figure 6. UPLC conditions were the same for all chromatograms. From the UPLC-MS chromatograms (Figure 6A), it was easily determined that Samples U2 and U3 were authentic black cohosh, and that Samples U 1 and U 4 were not. On the contrary, it was difficult to tell from the ELSD chromatograms (Figure 6B) which of the unknown samples was authentic black cohosh. Figure 6 clearly demonstrates that MS is a better technique for differentiating complex botanic samples than ELSD.

Conclusion

In this application note, a method for black cohosh authentication has been developed using the ACQUITY UPLC H-Class System and the ACQUITY QDa Mass Detector. Specifically, four black cohosh constituents: cimifugin, cimiracemoside C, 27-dexoyactein, and actein were screened, and cimiracemoside C was selected as a marker. A new automated data processing protocol that is suitable for routine authenticity testing was developed. In this data processing protocol, the marker's chromatographic and mass spectral data were evaluated for authenticity. This authenticity method was tested with four commercial black cohosh samples and two homemade contaminated NA black cohosh samples. All authentic and inauthentic or contaminated samples were correctly identified. It should be noted that due to the limited number of training or reference samples used in the method development, this black cohosh authenticity method may need further improvement or validation.

This application note demonstrates that the ACQUITY QDa Mass Detector is well suited for botanical authentication, which is an essential test for product safety and quality. The cimiracemoside C ion chromatogram collected by mass detection had less interfering peaks, and showed clear differences between the NA and Asian black cohosh samples. The cimiracemoside C mass spectrum contained a characteristic mass spectral pattern that was used for authenticity testing. The ACQUITY QDa is simple to use and affordable, and it can be easily implemented in analytical QC labs for routine botanical authentication. The adoption of mass detection for authenticity testing can greatly improve the speed and confidence for botanical authentication, and help to ensure the safety and high quality of dietary supplements, herbal medicines, cosmetics, and personal care products on the market.

References

1. S Gafner. Laboratory guidance documents, ABC-AHP-NCNPR botanical adulterants program. (2015). www.botanicaladulterants.org <http://www.botanicaladulterants.org/> . Acessed December 12, 2017.
2. P Geng, J M Harnly, J Sun, M Zhang, P Chen. Feruloyl dopamine-O-hexosides are efficient marker compounds as orthogonal validation for authentication of black cohosh *(acetaea racemosa)* – a UHPLC-HRAM-MS chemometrics study. *Anal Bioanal Chem.* (2017) Apr;409(10):2591–2600.
3. K He, G F Pauli, B Zheng, H Wang, N Bai, T Peng, M Roller, and Q Zheng. Cimicifuga species identification by high performance liquid chromatography-photodiode array/mass spectrometric/evaporative light scattering detection for quality control of black cohosh products. *J Chromatogr A.* (2006) April 21; 1112(1–2): 241–254.

4. M Sharaf, J Yuk, K Yu, M Wrona, G Isaac. Chemical Profiling of *Actaea* Species and Commercial Products Using UPLC-QTof-MS, Waters Posters presented at 9th Joint Natural Products Conference, July 24–27, 2016, Copenhagen, Denmark.

5. S Masada. Authentication of the botanical origin of Western herbal products using Cimicifuga and Vitex products as examples. *J Nat Med.* (2016) 70:361–375.

6. B Avula, Y-H Wang, T J Smillie, I A Khan. Quantitative determination of triterpenoids and formononetin in Rhizomes of black cohosh *(Actaea racemosa)* and dietary supplements by using UPLC-UV/ELS detection and identification by UPLC-MS. *Planta Med.* (2009) 75:381–386.

7. B Jiang, F Kronenberg, P Nuntanakorn, M-H Qiu, E J Kennelly. Evaluation of the botanical authenticity and phytochemical profile of black cohosh products by high-performance liquid chromatography with selected ion monitoring liquid chromatography– mass spectrometry. *J Agric Food Chem.* (2006) May 3; 54(9): 3242–3253.

8. P W Whiting, A Clouston, P Kerlin. (2002) Black cohosh and other herbal remedies associated with acute hepatitis. *Med J Aust.* 177:432–435.

9. J Yang, P Rainville. Automated 2-Dimensional Marker's Fingerprint Analysis for Routine Botanical Authentication Using the ACQUITY QDa Mass Detector. Waters application note no 720006284en < https://www.waters.com/waters/library.htm?&cid=134864024&lid=134981491> , May, 2018.

10. U.S. Pharmacopoeia-National Formulary [USP 40 NF 35]. Rockville, Md: United States Pharmacopeial Convention; 2017. http://app.uspnf.com/uspnf/pub/indexusp=40&nf=35&s=2&officialOn=December%201,%202017. < http://app.uspnf.com/uspnf/pub/index?usp=40&nf=35&s=2&officialOn=December%201,%202017> Accessed December 12, 2017.

Featured Products

ACQUITY UPLC H-Class PLUS System <https://www.waters.com/10138533>

ACQUITY QDa Mass Detector <https://www.waters.com/134761404>

Empower 3 Chromatography Data Software <https://www.waters.com/513188>

720006247, May 2018