



Automated 2-Dimensional Fingerprint Analysis for Routine Botanical Authentication Using the ACQUITY QDa Mass Detector

Jinchuan Yang, Paul D.Rainville

Waters Corporation

Abstract

In this application note, a simple 2-dimensional fingerprint analysis method is presented for NA black cohosh authenticity testing. The data processing was automatically handled by Empower CDS Software, which is convenient for routine analysis. The details of this 2-dimensional fingerprint analysis using Empower is described in this note.

Benefits

- Streamlined Empower Chromatography Data Software (CDS) processing is suitable for routine analysis
 - Simple and affordable MS detection for routine analyses
 - Highly selective and distinctive MS data is suitable for botanical authentication
-

Introduction

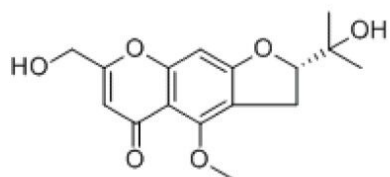
Botanical ingredients are widely used in dietary supplements, herbal medicines, cosmetics and personal care products. Potential contamination or misidentification of plants due to the lack of standardization of production has been a health concern to consumers. Ascertaining the authenticity of botanical ingredients and processed products is a challenging task due to their complex phytochemical constituents, the natural variation in their phytochemical profiles, and the similar phytochemical profiles of closely related species. Liquid chromatography (LC) coupled with mass spectrometry (MS) is one of the most effective analytical techniques¹⁻⁷ for authenticity evaluation. However, LC-MS has not been widely used in analytical labs for routine authenticity testing due to the relatively high cost of mass spectrometers and the high level of expertise needed. Recently, we have demonstrated the feasibility of using Waters ACQUITY UPLC H-Class System with the ACQUITY QDa Mass Detector for botanical authenticity testing.⁸ The distinctive MS ion chromatogram and the specific mass spectrum for a marker compound were found to be very effective in differentiating North American (NA) black cohosh (*Actaea racemosa*) from other *Actaea* species.

Chemometric analysis techniques, such as Principle Component Analysis (PCA), Similarity Analysis, and Clustering Analysis are powerful data processing tools for the authentication and classification of botanicals.⁹ However, data processing for these techniques is often sophisticated and time-consuming. In this application note, a simple 2-dimensional fingerprint analysis method is presented for NA black cohosh authenticity testing. The data processing was automatically handled by Empower CDS Software, which is convenient for routine analysis. The details of this 2-dimensional fingerprint analysis using Empower is described in this note.

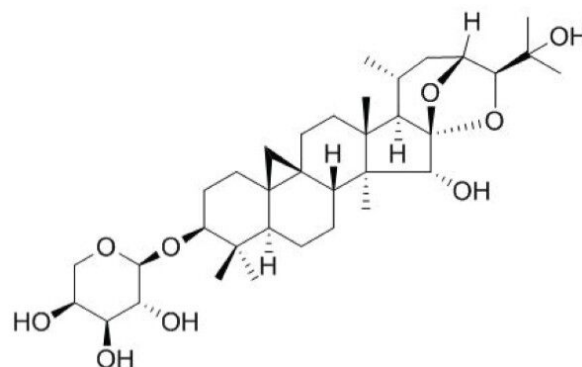
Experimental

Samples

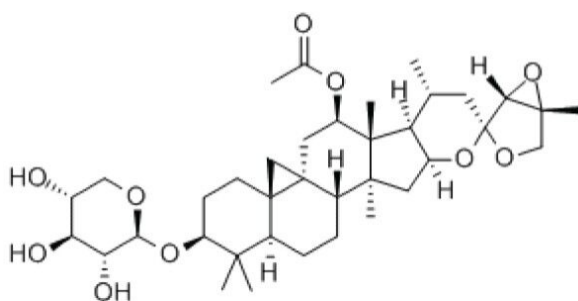
Three authentic NA black cohosh extracts (NA1-NA3), three Asian black cohosh (*Actaea cimicifuga*) extracts (A1-A3), and four commercial black cohosh samples (U1-U4) were provided by a collaborator. These extracts were diluted with 70% methanol to about 5 mg/mL. Four standards: cimifugin, cimiracemoside C (cimigenol-3- α -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 $\mu\text{g/mL}$. The standards' structures, CAS Registry numbers, and monoisotopic masses are shown in Figure 1. Home-made 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 μm PTFE membrane prior to the analysis.



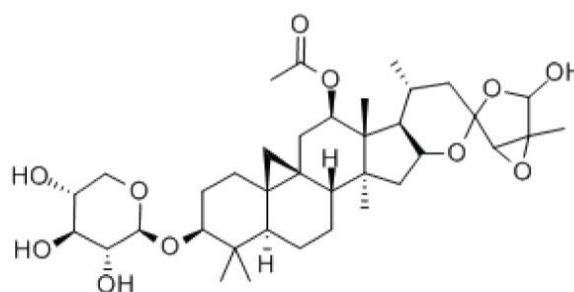
Cimifugin
 CAS:37921-38-3,
 monoisotopic mass 306.11 Da



Cimiracemoside C
 CAS: 256925-92-5,
 monoisotopic mass: 620.39 Da



27-deoxyactein
 CAS: 264624-38-6,
 monoisotopic mass: 660.39 Da



Actein
 CAS: 18642-44-9,
 monoisotopic mass: 676.38 Da

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

UPLC conditions

UPLC system:	ACQUITY UPLC H-Class
Column:	ACQUITY BEH C ₁₈ , 130Å, 1.7 μm, 2.1 mm x 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
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

Results and Discussion

Quantitative parameters for authentication

In the black cohosh authenticity study,⁸ cimracemoside C was selected as the marker for the authentic black cohosh. The extracted ion chromatograms (XICs) at the marker's molecular ion mass-to-charge ratio (m/z 621 Da) from the NA black cohosh samples shared a simple and consistent pattern that was significantly different from the XICs from the Asian black cohosh samples (Figure 2). In addition, the mass spectra extracted at the marker's peak retention time (RT) 5.77 min showed a characteristic pattern, as shown in Figure 3. These patterns, or fingerprints, from two orthogonal dimensions provided the basis for this NA black cohosh authentication method.

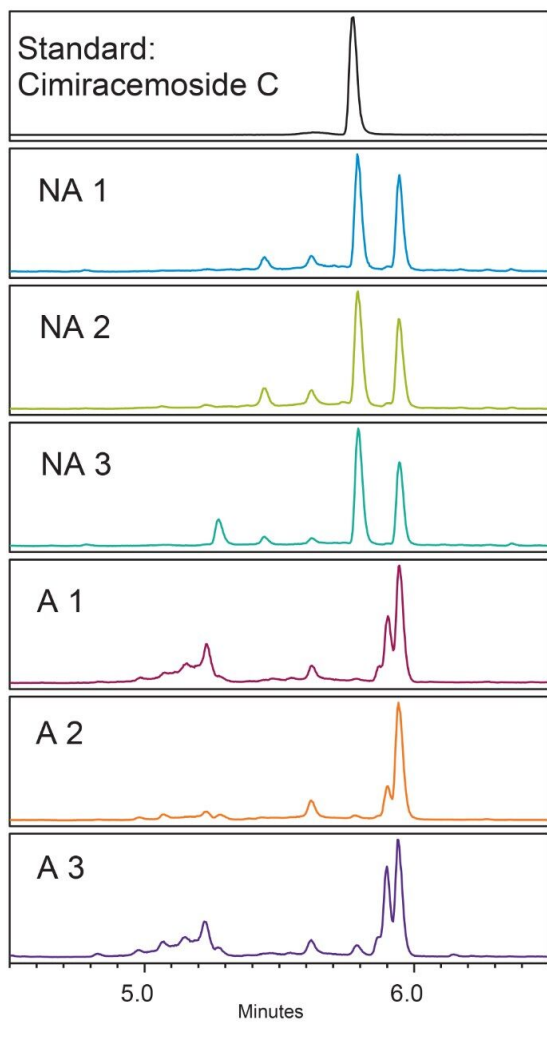


Figure 2. Extracted ion chromatograms of cimracemoside C (m/z 621 Da) in standards, NA black cohosh (NA 1-NA 3), and Asian black cohosh (A 1-A 3) samples.

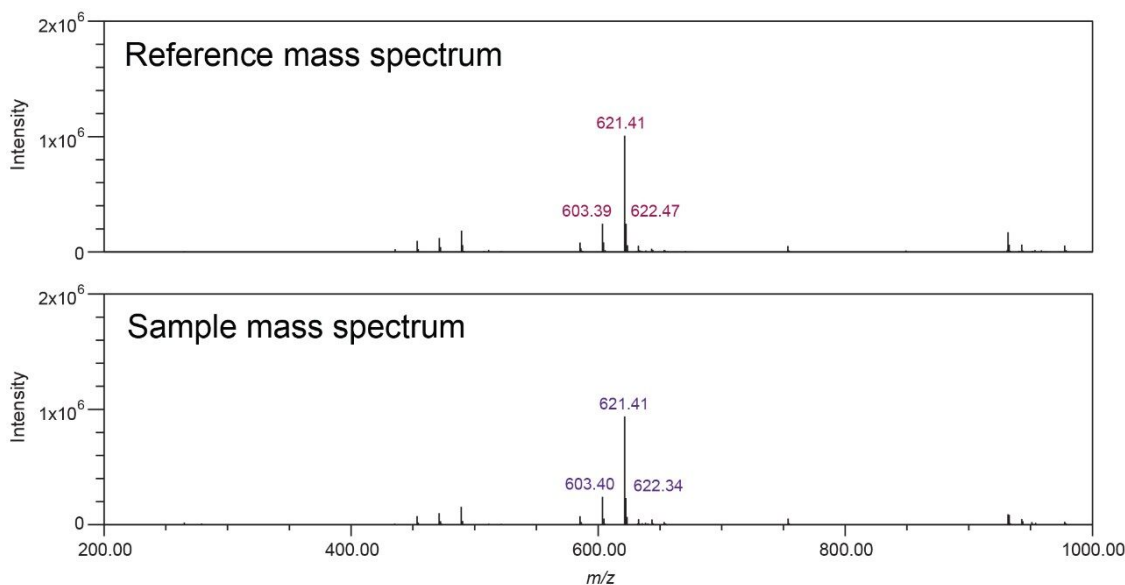


Figure 3. Empower Library Match results. The mass spectrum of the marker (*cimiracemoside C*) peak in a sample (bottom) matches one of the reference mass spectra in the NA black cohosh MS library (top).

Chromatographic pattern

The common feature in the XICs of the NA black cohosh was that there were two main peaks of about equal peak height, and the marker's peak was one of them (Figure 2, NA 1-NA 3). We used the marker peak's RT and its peak relative area as the quantitative parameters to characterize this chromatographic pattern. Additional parameters could be used, but these two parameters seemed to be effective enough to differentiate the NA black cohosh from the Asian black cohosh samples.

Mass spectral pattern

The top five abundant ions in the extracted mass spectra from NA black cohosh samples were used to characterize the pattern. These ions include the molecular ion (base peak, m/z 621 Da), a fragment ion (m/z 603 Da), the sodium adduct ion (m/z 643 Da), and the isotopic ions (m/z 622, 644 Da). Their m/z and relative intensity (relative to the base peak, or the molecular ion) values were used as the quantitative parameters for authentication. It should be noted that these mass spectra were obtained from the NA black cohosh samples, not from the *cimiracemoside C* standard. Therefore it is necessary to include those adduct and isotopic ions, in addition to the molecular ion and the fragment ion, in order to capture the overall spectral pattern of NA black cohosh samples at the UPLC RT 5.77 min.

MS Library Match

The extracted mass spectra (at RT 5.77 min) were stored in a customized NA black cohosh MS library, and were used for MS library search in the unknown black cohosh sample authenticity testing. One example of the Empower MS Library Match results is shown in Figure 3.

Threshold values for automated analysis

To determine the threshold values in those authentication parameters for the NA black cohosh, three NA black cohosh and three Asian black cohosh samples were measured in triplicate by UPLC-MS. These samples were measured in a randomized fashion to avoid artifacts related to injection order. Table 2 shows the statistical averages, the standard deviations (SD), and the threshold values for those authentication parameters. In Table 2, the upper and the lower limits in RT were set at the $\pm 1\%$ of the RT average. For the relative peak area (%Area), the limits were set at 3X the SD from the average. For the expected mass relative intensity, the lower limits were set at 3X the SD below the average. There was no upper limit used for the expected mass relative intensity. These threshold values were mainly chosen at the 3X the SD to cover the potentially wide variation in the NA black cohosh. A data processing Method Set was created in Empower Software to carry out the authentication process. Figure 4 shows the Empower data processing flow chart. Table 3 shows the Empower functions that were used in this method.

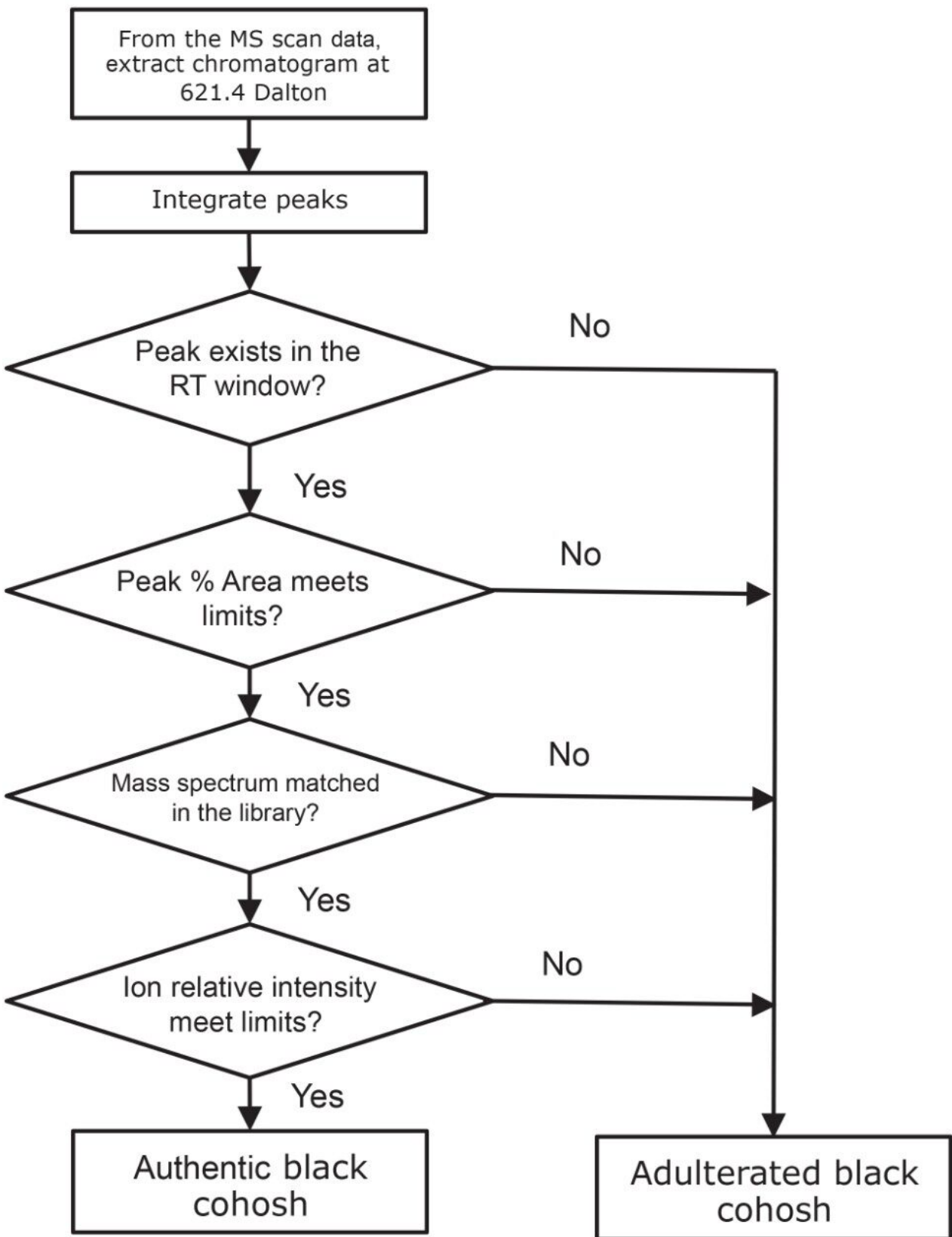


Figure 4. Authenticity data process protocol for black cohosh using a single marker's chromatographic pattern and its mass spectral pattern.

Parameters	Ion chromatogram pattern		Mass spectral pattern									
	RT (min) ⁺	%Area	Expected mass 1		Expected mass 2		Expected mass 3		Expected mass 4		Expected mass 5	
			m/z (Da)	Intensity (%)	m/z (Da)	Intensity (%)	m/z (Da)	Intensity (%)	m/z (Da)	Intensity (%)	m/z (Da)	Intensity (%)
Average	5.771	50.3	621	97	643	75	622	34	644	23	603	18
SD	0.005	3.3		6		22		5		5		3
Upper limit ⁺⁺	5.829	60.0		–		–		–		–		–
Lower limit	5.713	40.0		79		9		17		7		8

+: ±1% of the RT is used for the upper and lower limits.

++: Upper limits for mass spectral pattern are not used.

Table 2. Characteristics of the chromatographic pattern and the mass spectral pattern for NA black cohosh, and the threshold values in authentication parameters.

Authentication criteria	Empower functions and fields
	<ul style="list-style-type: none"> • Derived channel for XIC at 621.4 Dalton; • XIC peak integration
<ul style="list-style-type: none"> • Peak exist in the RT window? • Peak %Area meets the limits? 	<ul style="list-style-type: none"> • Peak found in the RT window that specified in the Component Table; • %Area within the component suitability limits.
<ul style="list-style-type: none"> • Mass spectrum matched in the MS library? • Ion relative intensity meet limits? 	<ul style="list-style-type: none"> • Spectrum found in the Empower MS Library search; • MS Expected Masses found, and the Expected Intensities are higher than the limits

+: ±1% of the RT is used for the upper and lower limits.

++: Upper limits for mass spectral pattern are not used.

Table 3. The NA black cohosh authentication criteria and the related Empower functions and fields used in the Empower data processing Method Set.

Analysis of commercial and home-made black cohosh samples

Four commercial black cohosh samples (U1-U4) and two home-made black cohosh samples (M5 and M10) were tested by this method. Figure 5 shows an Empower report for these samples. Any nonconformity to the authentication criteria was automatically flagged in red by Empower Software. A summary of the test results was discussed in the previous [application note](#) <

<https://www.waters.com/waters/library.htm?&cid=134528742&lid=134981488&locale=184>>.⁸ Briefly, the authentic, inauthentic, and contaminated black cohosh samples were all correctly determined.

Sample	Name	RT	% Area	Peak Type	MS Match1 Spect. Name	Expected Mass 1 (Da)	Expected Intensity 1 (%)	Expected Mass 2 (Da)	Expected Intensity 2 (%)	Expected Mass 3 (Da)	Expected Intensity 3 (%)	Expected Mass 4 (Da)	Expected Intensity 4 (%)	Expected Mass 5 (Da)	Expected Intensity 5 (%)
U1	<i>Black Cohosh</i>	5.773	5	Found	BlackCohosh#67	621.4	9	643.5	10						
U2	BlackCohosh	5.750	44	Found	BlackCohosh#67	621.5	100	643.5	50	622.5	30	644.5	18	608.4	20
U3	BlackCohosh	5.771	43	Found	BlackCohosh#66	621.4	100	643.5	68	622.5	38	644.5	26	608.5	21
U4	<i>Black Cohosh</i>	5.766	4	Found	BlackCohosh#66	621.4	49	643.5	28	622.4	15	644.4	10		
M5	<i>Black Cohosh</i>	5.773	48	Found	BlackCohosh#66	621.4	31	643.5	100	622.4	13	644.5	28	608.5	6
M10	<i>Black Cohosh</i>	5.774	59	Found	BlackCohosh#68	621.5	36	643.5	100	622.4	9	644.5	39	608.4	6

Figure 5. Screen shot of an Empower Software authenticity test report for the commercial and home-made samples.

Benefits of this novel authentication approach

Chemometric analyses are powerful tools for exploratory authenticity studies of botanicals. In the routine analysis environment, however, they are too sophisticated and cumbersome to be implemented. Here, we have demonstrated a novel authentication approach, in which a marker compound's 2-dimensional fingerprints are used to authenticate NA black cohosh. Because only the marker's chromatographic fingerprint and mass spectral fingerprint are processed, the amount of the data that need to be processed is relatively small, and the data handling is relatively simple. The whole data processing can be automated in Empower Software, which is suitable for laboratories performing routine analysis.

Conclusion

In this application note, details of an automated 2-dimensional fingerprint analysis for NA black cohosh authenticity are described. The key features, or patterns, in the chromatographic and the mass spectral fingerprints of a marker compound were characterized by a set of quantitative parameters, such as RT, peak relative area, m/z , and ion relative intensity. The threshold values of these parameters for NA black cohosh were determined and used in Empower Software's automated data processing. Using this UPLC-MS

approach, we were able to differentiate NA black cohosh from Asian black cohosh samples, and detect Asian black cohosh contamination at 5 wt%. It should be noted that due to the limited number of reference or training samples used in method development, this black cohosh authenticity method may need to be further validated.

The key features of this UPLC-MS approach include the use of the ACQUITY QDa Mass Detector, the use of a marker's 2-dimensional fingerprints for authentication, and the automation of the whole data processing by Empower Software. The ACQUITY QDa is affordable, easy to learn, and use. Automated data processing using Empower Software is quick and objective. These features are suitable for routine authenticity testing, where the analyst's time and expertise may be limited. This new UPLC-MS approach could be easily implemented in analytical labs for the routine authentication, and/or quality control of botanical ingredients and finished products in dietary supplements, herbal medicines, cosmetics and personal care products to safeguard product quality and safety.

References

1. S Gafner. Laboratory guidance documents, ABC-AHP-NCNPR botanical adulterants program.(2015); www.botanicaladulterants.org Accessed 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 (*actaea 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, 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 poster no. PST134905017 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 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.
7. B Avela, 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 using UPLC-UV/ELS detection and identification by UPLC-MS. *Planta Med.* (2009); 75:381–386.
8. J Yang, J Yuk, P Rainville. Benefits of Mass Detection using the ACQUITY QDa for Routine Botanical Authentication. Waters Application Note 720006247en, May 2018.
9. D Jing, W Deguang, H Linfang, C Shilin, Q Minjian. Application of chemometrics in quality evaluation of medicinal plants. *J Medicinal Plants Research.* (2011); Vol. 5(17):4001–4008.

Featured Products

[Empower 3 CDS Software <https://www.waters.com/10190669>](https://www.waters.com/10190669)

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

[ACQUITY QDa Mass Detector <https://www.waters.com/134761404>](https://www.waters.com/134761404)

720006284, May 2018