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Application News

High Performance Liquid Chromatograph Nexera™series

Qualitative Determination of Abietic Acid Improperly Used in Prepared Chinese Medicines

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User Benefits

- ◆ Nexera™ can analyze abietic acid with a high sensitivity, good consistency, and highly accurate results.
- ◆ It is possible to evaluate drug quality by the similarity of UV spectra.

■ Introduction

A method of testing for abietic acid and determining its UV spectrum in prepared Chinese medicines using high-performance liquid chromatograph Nexera series was devised based on supplementary methods of testing for abietic acid (BJY 201908, BJY 201909, BJY 201919, BJY 202004, and BJY 202005) issued by the National Medical Products Administration. Results of an experiment revealed that the theoretical plate number calculated based on the peak for abietic acid was in accordance with the supplementary methods of testing for abietic acid. Abietic acid was not detected in any of the following prepared Chinese medicines: *Nujin* pills, *Jiebai* capsules, *Chenxiang Huazhi* pills, *Fengshi Ershiwuwei* pills, or *Shensanqi Shangyao* capsules. Nexera is an entirely new generation of high-performance liquid chromatograph with a high sensitivity, good consistency, and highly accurate results, and is suitable for tasks related to drug quality control in the pharmaceutical industry and industries involving drug testing.

Abietic acid is a hard and brittle glassy solid with an odor of pine resin. It has an extremely wide range of applications and is used in fields such as rubber, plastics, paints, paper-making, surfactants, pharmaceuticals, electrical devices, and building materials. In adhesives, it is mainly used as a tackifier. A mixture of rosin dust and air is explosive. Abietic acid has limited toxicity but its concentrated vapor can cause symptoms of acute poisoning such as headaches, dizziness, coughing, and asthma.

The National Medical Products Administration has issued a number of bulletins on supplementary methods of testing for abietic acid (BJY 201908, BJY 201909, BJY 201919, BJY 202004, and BJY 202005) that specify methods of testing for abietic acid in relevant prepared Chinese medicines. Liquid chromatography is used to qualitatively determine its retention time and photodiode array (PDA) spectral information in prepared Chinese medicines.

Based on the supplementary methods of testing for abietic acid mentioned above, a method of using a liquid chromatograph equipped with photodiode array detector to test for abietic acid improperly used in five traditional Chinese medicines was devised. This method integrates chromatographic separation and qualitative aspects of UV spectra, offers a high selectivity and sensitivity, and can provide a reference for related industries.

■ Experiment

System

Nexera was used in this experiment and configured as follows:

Fig.1 Shimadzu Nexera™

Table 1 System configuration

Solvent Delivery Pump	:	LC-40B XR
Autosampler	:	SIL-40C XR
Column oven	:	CTO-40C
On-Line Degassing Unit	:	DGU-405
System Controller	:	SCL-40
Detector	:	SPD-M40
Chromatography Workstation	:	LabSolutions Ver 5.97

Analytical Conditions

Table 2 Analytical Conditions

Column	:	Shim-pack™ VP-ODS* (150 mm × 4.6 mm I.D., 5 µm)
Mobile Phase A	:	0.1 % Formic acid
Mobile Phase B	:	Acetonitrile
Flow Rate of Mobile Phase	:	1 mL/min
Column temp.	:	40 ℃
Wavelength	:	241 nm (SPD-M40 Standard cell)
Injection Volume	:	10 µL (*Chenxiang Huazhi* pills: 20 µL)
Elution Mode	:	*Nujin* pills and *Jiebai* capsules: Isocratic elution, B phase concentration of 80 %; *Fengshi Ershiwuwei* pills and *Shensanqi Shangyao* capsules: Isocratic elution, B phase concentration of 75 %; *Chenxiang Huazhi* pills: Gradient elution. The gradient is shown in Table 3, and B phase initial concentration of 60 %;

* P/N: 228-34937-91

Table 3 Time Program for *Chenxiang Huazhi* Pills

Time (min)	Unit	Processing Command	Value
30.00	Pump	B.Conc	70
35.00	Pump	B.Conc	65
35.01	Pump	B.Conc	60
40.00	Controller	Stop	

Preparation of the Control Solutions

***Nujin* pills**: (Freshly prepared for immediate use) Precisely weigh an appropriate amount of the abietic acid control and add 50 % methanol to make a solution containing 2 μg per 1 mL to serve as the control solution. Precisely weigh an appropriate amount of the 3-Acetyl-11-keto-β-Boswellic acid control, add 50 % methanol to make a solution containing 2 μg per 1 mL to serve as the reference solution. (Refer to BJY 201908, a supplementary method of testing for abietic acid.)

***Jiebai* capsules**: (Freshly prepared for immediate use) Add ethanol to an appropriate amount of the abietic acid control to make a solution containing 10 μg per 1 mL. (Refer to BJY 201909, a supplementary method of testing for abietic acid.)

***Chenxiang Huazhi* pills, *Fengshi Ershiwuwei* pills, *Shensanqi Shangyao* capsules:** (Freshly prepared for immediate use) Precisely weigh an appropriate amount of the abietic acid control and add ethanol to make a solution containing 2 μg per 1 mL. Precisely weigh an appropriate amount of the 3-Acetyl-11-keto-β-Boswellic acid control and add ethanol to make a solution containing 2 μg per 1 mL to serve as the reference solution. (Refer to BJY 201919, BJY 202004, and BJY 202005, three supplementary methods of testing for abietic acid.)

Test Sample Preparation

***Nujin* pills:** Add 4.5 g of diatomaceous earth to 9 g of this product and finely grind. Precisely weigh 3 g of the mixture, add 20 mL of 50 % methanol, sonicate for 30 minutes, and filter. Precisely measure 1 mL of the filtrate, place it in a 10-mL volumetric flask, and dilute with 50 % methanol dilute to the mark. Shake well.

***Jiebai* capsules:** Finely grind an appropriate amount of the capsule contents. Add 20 mL of ethanol to 0.2 g of the ground mixture and sonicate for 20 minutes. Allow the liquid mixture to come to room temperature. Filter the supernatant through a microfiltration membrane (0.45 μm) to obtain the filtrate.

***Chenxiang Huazhi* pills:** Finely grind this product. Precisely weigh 2.0 g and add 20 mL of ethanol. Weigh the mixture and then sonicate it for 30 minutes. Allow it to cool and weigh it again. Make up any loss in weight with ethanol. Shake well and filter to obtain the filtrate.

***Fengshi Ershiwuwei* pills and *Shensanqi Shangyao* capsules:** Finely grind each product. Precisely weigh 0.2 g and add 20 mL of ethanol. Weigh the mixture and then sonicate it for 20 minutes. Allow it to cool and weigh it again. Make up any loss in weight with ethanol. Shake well and filter to obtain the filtrate.

■ Results and Discussion

Basis for Interpretation of the Analytical Results

***Jiebai* capsules:** A peak should not appear in the chromatogram for the test sample at a position corresponding to the retention time of the peak for the abietic acid control solution. If a peak with the same retention time is evident, then the diode array detector will be used to compare the UV-visible absorption spectrum of the corresponding peak. The absorption spectra should differ. (The peak for the abietic acid control displays maximum absorbance at 241 nm.) If the absorption spectra are the same, then abietic acid is deemed to be detected (supplementary method of testing for abietic acid BJY 201909 from the National Medical Products Administration).

***Nujin* pills, *Chenxiang Huazhi* pills, *Fengshi Ershiwuwei* pills, and *Shensanqi Shangyao* capsules:** A peak should not appear in the chromatogram for the test sample at a position corresponding to the retention time of the peak for the abietic acid control solution. If a peak with the same retention time is evident, then the diode array detector will be used to compare the UV-visible absorption spectrum of the corresponding peak. The absorption spectra should differ. (The peak for the abietic acid control displays maximum absorbance at 241 nm.) If the absorption spectra are the same and the peak area is larger than the peak area for the 3-Acetyl-11-keto-β-Boswellic acid reference solution, then abietic acid is deemed to be detected (supplementary methods of testing for abietic acid BJY 201908, BJY 201919, BJY 202004, and BJY 202005 from the National Medical Products Administration).

Results for the Test Samples

***Nujin* pills:** The control solution, reference solution, and sample solution were tested in accordance with Analytical Conditions. Chromatograms are shown in Fig.2 and test results are shown in Table 4. Results revealed that the chromatogram for the sample solution lacked a peak at the same position corresponding to the retention time of the peak for the abietic acid control solution. Thus, abietic acid was not detected in *Nujin* pills and results conformed to regulations. In order to demonstrate the helpful qualitative features of UV spectra, abietic acid was added to the test sample to prepare a spiked test sample, its UV spectra were qualitatively analyzed. The spectra and spectral similarity are shown in Fig.3 Spectral similarity was 0.9824.

***Jiebai* capsules:** The control solution, reference solution, and sample solution were tested in accordance with Analytical Conditions. Chromatograms are shown in Fig.4 and test results are shown in Table 5. Results revealed that the chromatogram for the sample solution lacked a peak at the same position corresponding to the retention time of the peak for the abietic acid control solution. Thus, abietic acid was not detected in *Jiebai* capsules and results conformed to regulations. The spectrum for and spectral similarity of a spiked test sample are shown in Fig.5. Spectral similarity was 0.9947.

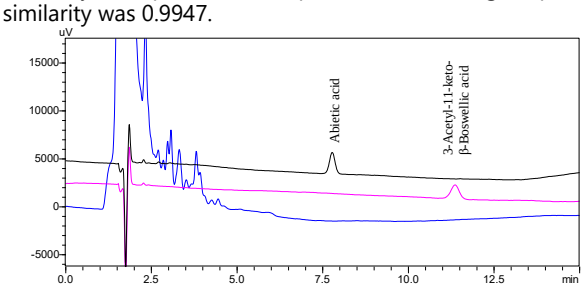


Fig. 2 Chromatograms for the Control Solution, Reference Solution, and Sample Solution (*Nujin* pills)
Black: Abietic acid; Magenta: 3-Acetyl-11-keto-β-Boswellic acid; Blue: *Nujin* pills;

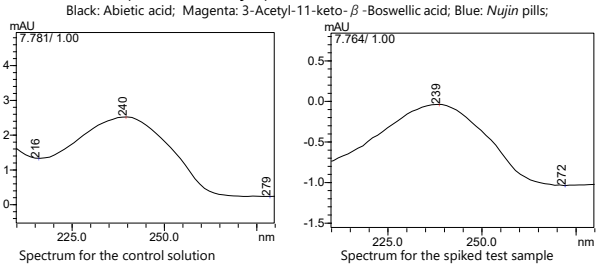


Fig.3 Spectra for the Control Solution and the Spiked Test Sample (*Nujin* pills)

Table 4 Test Results for the Control Solution and Sample Solution (*Nujin* pills)

Name of Solution	Retention Time (min)	Theoretical Plate Number	Theoretical Plate Number (required for the method of testing)
Abietic acid (control solution)	7.779	9811	NLT 2000
3-Acetyl-11-keto-β-Boswellic acid (reference solution)	11.371	/	/
Abietic acid (sample solution)	N.D.	/	/

N.D. = Not detected

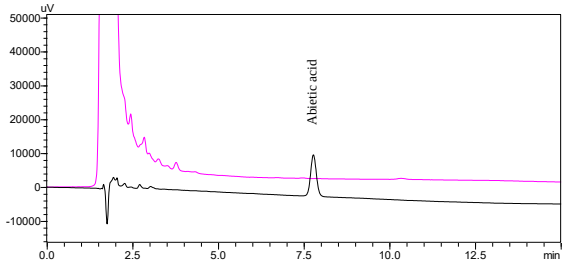


Fig. 4 Chromatograms for the Control Solution and Sample Solution (*Jiebai* capsules) Black: abietic acid; Magenta: *Jiebai* capsules;

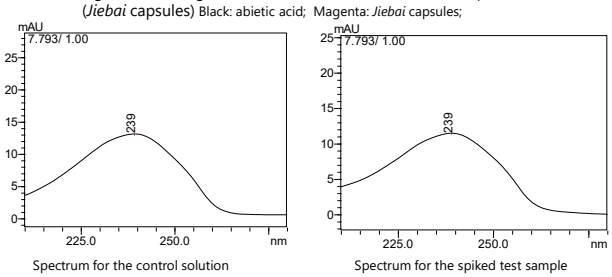


Fig.5 Spectra for the Control Solution and the Spiked Test Sample (*Jiebai* capsules)

Table 5 Test Results for the Control Solution and Sample Solution (*Jiebai* capsules)

Name of Solution	Retention Time (min)	Theoretical Plate Number	Theoretical Plate Number (required for the method of testing)
Abietic acid (control solution)	7.786	9499	NLT 2000
Abietic acid (sample solution)	N.D.	/	/

N.D. = Not detected

Chenxiang Huazhi pills: The control solution, reference solution, and sample solution were tested in accordance with Analytical Conditions. Chromatograms are shown in Fig.6 and test results are shown in Table 6. Results revealed that the chromatogram for the sample solution had a suspected peak for abietic acid at the position corresponding to the retention time of the peak for the abietic acid control solution. A spectrum was obtained and spectral similarity was determined. (Results are shown in Fig.7.) Spectral similarity was 0.6878. Thus, abietic acid was not detected in *Chenxiang Huazhi* pills and results conformed to regulations. The spectral similarity of the spiked test sample was 0.9939.

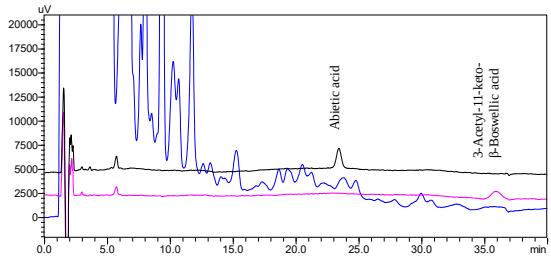


Fig.6 Chromatograms for the Control Solution, Reference Solution, and Sample Solution (*Chenxiang Huazhi* pills)
Black: Abietic acid; Magenta: 3-Acetyl-11-keto-β-Boswellic acid; Blue: *Chenxiang huazhi* pills;

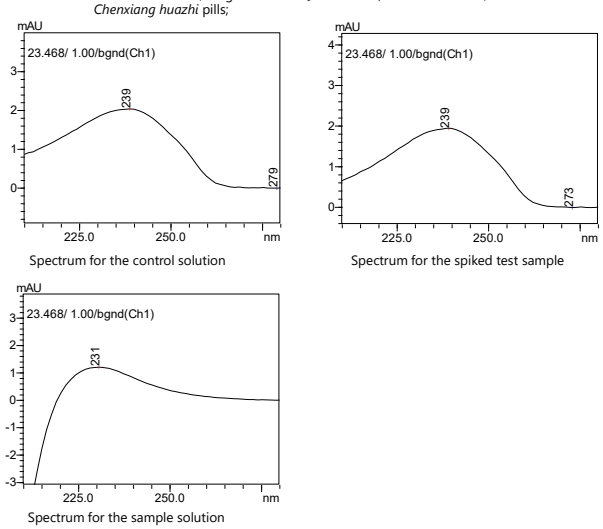


Fig.7 Spectra for the Control Solution, the Spiked Test Sample and Sample solution (*Chenxiang Huazhi* pills)

Table 6 Test Results for the Control Solution and Sample Solution (*Chenxiang Huazhi* pills)

Name of Solution	Retention Time (min)	Theoretical Plate Number	Theoretical Plate Number (required for the method of testing)
Abietic acid (control solution)	23.459	16295	NLT 2000
3-Acetyl-11-keto-β-Boswellic acid (reference solution)	35.932	/	/
Abietic acid (sample solution)	N.D.	/	/

N.D. = Not detected

***Fengshi Ershiwuwei* pills:** The control solution, reference solution, and sample solution were tested in accordance with Analytical Conditions. Chromatograms are shown in Fig.8 and test results are shown in Table 7. Results revealed that the chromatogram for the sample solution lacked a peak at the same position corresponding to the retention time of the peak for the abietic acid control solution. Thus, abietic acid was not detected in *Fengshi Ershiwuwei* pills and results conformed to regulations. The spectrum for and spectral similarity of the spiked test sample are shown in Fig.9. Spectral similarity was 0.9802.

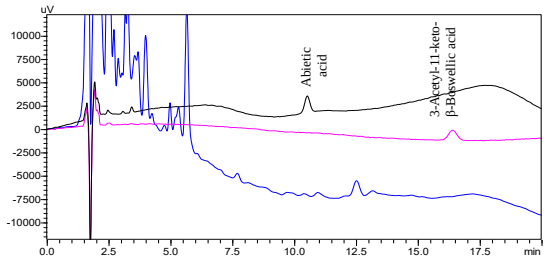
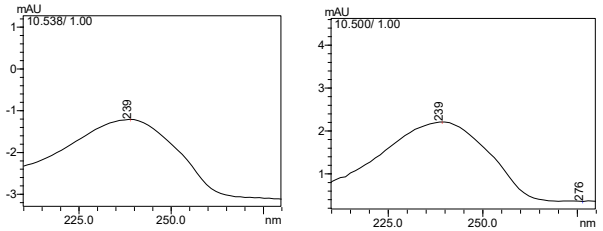


Fig.8 Chromatograms for the Control Solution, Reference Solution, and Sample Solution (*Fengshi Ershiwuwei* pills)
Black: Abietic acid; Magenta: 3-Acetyl-11-keto-β-Boswellic acid; Blue: *Fengshi Ershiwuwei* pills;



Spectrum for the control solution　　Spectrum for the spiked test sample

Fig 9 Spectra for the Control Solution and the Spiked Test Sample (*Fengshi Ershiwuwei* pills)

Table 7 Test Results for the Control Solution and Sample Solution (*Fengshi Ershiwuwei* pills)

Name of Solution	Retention Time (min)	Theoretical Plate Number	Theoretical Plate Number (required for the method of testing)
Abietic acid (control solution)	10.505	9481	NLT 3000
3-Acetyl-11-keto-β-Boswellic acid (reference solution)	16.400	/	/
Abietic acid (sample solution)	N.D.	/	/

N.D. = Not detected

***Shensanqi Shangyao* capsules:** The control solution, reference solution, and sample solution were tested in accordance with Analytical Conditions. Chromatograms are shown in Fig.10 and test results are shown in Table 8. Results revealed that the chromatogram for the sample solution lacked a peak at the same position corresponding to the retention time of the peak for the abietic acid control solution. Thus, abietic acid was not detected in *Shensanqi Shangyao* capsules and results conformed to regulations. The spectrum for and spectral similarity of the spiked test sample are shown in Fig.11. Spectral similarity was 0.9941.

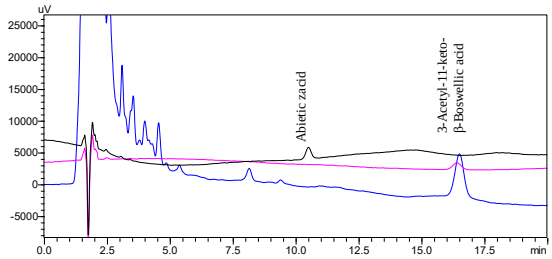
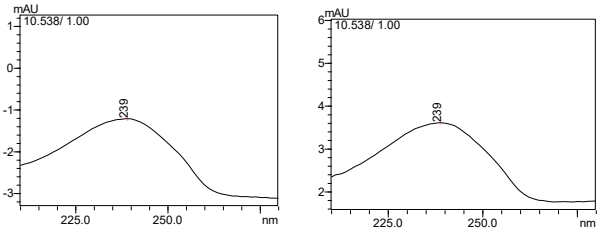


Fig. 10 Chromatograms for the Control Solution, Reference Solution, and Sample Solution (*Shensanqi Shangyao* capsules)
Black: Abietic acid; Magenta: 3-Acetyl-11-keto-β-Boswellic acid; Blue: *Shensanqi Shangyao* capsules;



Spectrum for the control solution　　Spectrum for the spiked test sample

Fig.11 Spectra for the Control Solution and the Spiked Test Sample (*Shensanqi Shangyao* capsules)

Table 8 Test Results for the Control Solution and Sample Solution (*Shensanqi Shangyao*capsules)

Name of Solution	Retention Time (min)	Theoretical Plate Number	Theoretical Plate Number (required for the method of testing)
Abietic acid (control solution)	10.508	10159	NLT 3000
3-Acetyl-11-keto-β-Boswellic acid (reference solution)	16.404	/	/
Abietic acid (sample solution)	N.D.	/	/

N.D. = Not detected

■ Conclusion

Nexera was used to qualitatively determine abietic acid improperly used in five traditional Chinese medicines based on supplementary methods of testing for abietic acid. This method integrates chromatographic separation and qualitative aspects of UV spectra, offers a high selectivity and sensitivity, and can be used as a reference for testing in related industries.

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