Analysis of Saponins in Spine Date Seed using Agilent ZORBAX Solvent Saver HT (1.8 µm) Columns

Application Note

Food and Traditional Chinese Medicine

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Abstract

The two main active compounds in spine date seed, the saponins Jujuboside A and B, were extracted and analyzed by high performance liquid chromatography (HPLC) with Agilent ZORBAX Eclipse Plus C18 LC columns. The method was first developed on a traditional 4.6 mm × 150 mm, 5 µm column and then transferred to a ZORBAX Eclipse Plus Solvent Saver High Throughput (1.8 µm) C18 column, 3.0 mm × 50 mm, 1.8 µm. The advantages of the Solvent Saver HT column include low solvent consumption and faster analysis. An evaporative light scattering detector (ELSD) was used due to the lack of chromophores in the two compounds. This provided higher sensitivity and a cleaner baseline compared with UV detection.

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Introduction

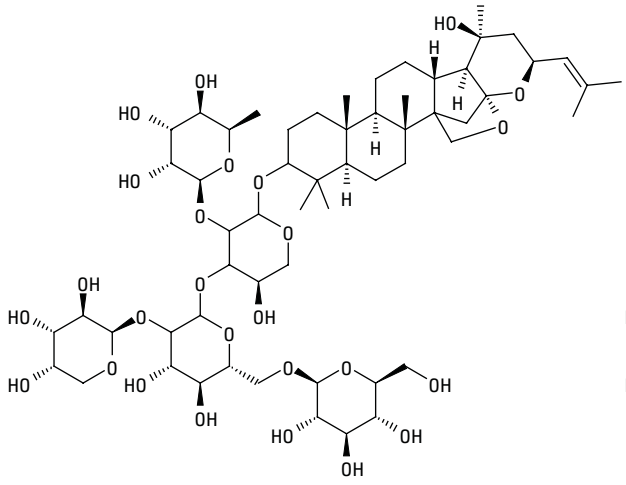
Jujube has been a part of food and Chinese medicines for at least 2,500 years. The Chinese used it as a Qi tonic to strengthen liver function. Jujube has a pleasant taste as well as a high nutritional value. The fruit contains vitamins A, B2, and C, and saponins, flavonoids, sugars, mucilage, calcium, phosphorus, and iron. Europeans have recognized its usefulness as a tonic for all parts of the body, especially the lungs and kidneys even though since the 17th century, the West dismissed Jujube as not having any medicinal qualities.[1]

Jujube seeds, or the English name spine date seeds, are used for medicinal purposes. Saponin compounds are also present in many plants such as Ginseng and Notoginseng, and have been the subject of research for a long time. The saponin compounds have a triterpenes structure, and many other triterpenes have been proven to have therapeutic effects.

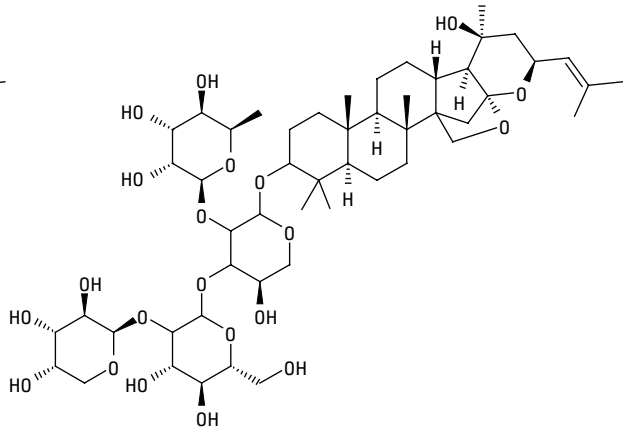
Scientific studies with animals have shown Jujubosides to have anti-anxiety and hypnotic effects, causing a reduction in the speed of conditioned reflexes, a reduction in hyperactivity, and a lowering of blood pressure. [1] Therefore, they can be used as a remedy for irritability, insomnia, anxiety, oedema, congestive heart failure, asthma and throat diseases.

The structures of the two main saponin compounds in spine date seeds, Jujubosides A and B, are shown in Figure 1. Since these compounds lack a chromophore and do not produce a sufficient signal with an ultraviolet (UV) detector, the ELSD which is a universal detector, was used in this method.

In this application note, the separation of the Jujobosides on the ZORBAX Solvent Saver RRHT column (3.0 mm × 50 mm, 1.8 µm) was compared to the separation on a traditional ZORBAX column (4.6 mm × 150 mm, 5 µm).



Jujuboside A MW 1207.36



Jujuboside B MW 1045.22

Figure 1. Structures of Jujubosides.

Experimental

Sample preparation

Spine date seeds were purchased at a pharmacy (Qun-Li TCM Pharmacy). The sample was treated as follows to extract the target compounds for analysis. [2]

- Weigh 5.0 g of the dried powder.
- Degrease with Soxhlet extractor using 50 mL ethyl ether for 4 h.
- Filter after cooling down and discard the liquid phase, then dry the residues.
- Put the residues in a 250-mL round-bottom flask, add 50 mL of methanol and extract for 2 h.
- Repeat and mix the extracts.
- Recover the methanol under vacuum and add 20 mL of water to the residue to dissolve.
- Extract using 15 mL of water saturated n-Butanol 3 times, then mix the extracts and evaporate under vacuum.
- Dissolve the residue with up to 5.0 mL methanol. Filter this final sample with a 0.45-µm Regenerated Cellulose Membrane filter (p/n: 5064-8221) before injecting into HPLC for analysis.

HPLC conditions

The HPLC analysis was performed with the Agilent 1200 Series Rapid Resolution LC (RRLC) system including a G1312B binary pump SL, G1376C automatic liquid sampler SL (ALS), G1316B Thermostated Column Compartment SL (TCC), G1316C Diode Array Detector SL (DAD) and G4218A Evaporative Light Scattering Detector (ELSD).

Mobile Phase:	35% water, 65% methanol
Flow rate:	1mL/min for 4.6 mm × 150 mm, 5 µm; 0.4 mL/min for 3.0 mm × 50 mm, 1.8 µm
Injection volume:	5 µL for 4.6 mm × 150 mm, 5 µm; 2 µL for 3.0 mm × 50 mm, 1.8 µm
Column:	ZORBAX Eclipse Plus C18, 4.6 mm × 150 mm, 5 µm (p/n: 959993-902) and 3.0 mm × 50 mm, 1.8 µm (p/n: 959941-302)
UV:	210 nm
TCC temp:	30 °C
ELSD temp:	40 °C
ELSD pressure:	3.5 bar
ELSD gain:	7
ELSD filter:	5s for 4.6 mm × 150 mm and 2s for 3.0 mm × 50 mm

Results and Discussion

The two compounds were separated well on the ZORBAX Eclipse Plus C18 column (4.6 mm × 150 mm, 5 µm) with excellent peak shape using ELSD detection as shown in Figure 2. For this complex sample matrix, many of the potential compounds have absorption below 210 nm, which made it difficult to get a clean baseline. The two target peaks detected at UV 210 nm were not well resolved from matrix compounds, which results in a potential error in quantitative results. The ELSD gave higher sensitivity compared to UV detection and a cleaner baseline with fewer matrix analytes. The two peaks of interest were baseline resolved.

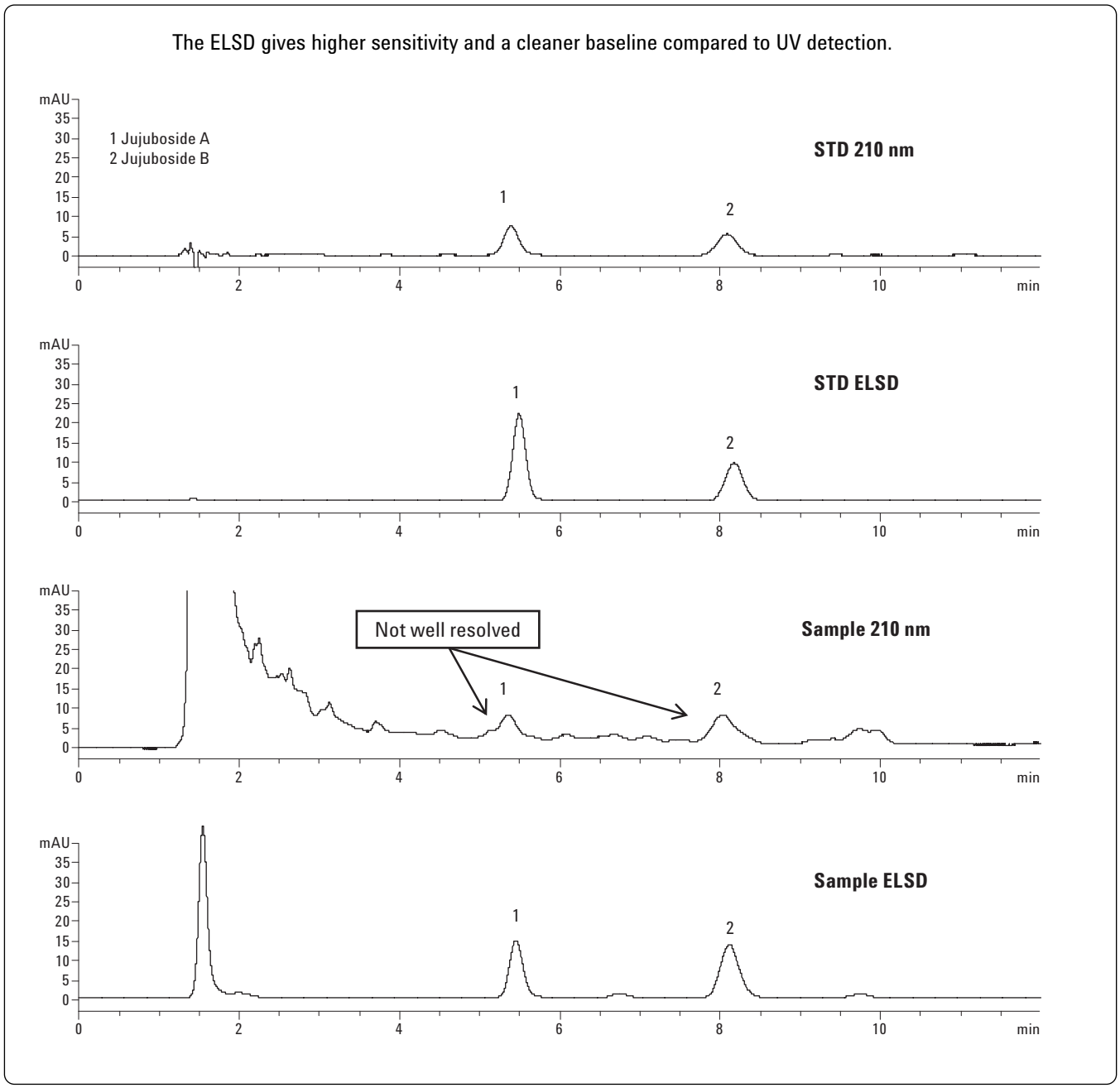


Figure 2. Standards and sample chromatograms by UV 210 nm/ELSD on Agilent ZORBAX Eclipse Plus C18, 4.6 mm × 150 mm, 5 µm.

The method developed in this analysis is a conventional method with about a 12-min analysis time and 12 mL of solvent consumption in one injection. The method can be easily adapted to a Solvent Saver HT column to achieve a savings in time and the amount of solvent used (Figure 3).

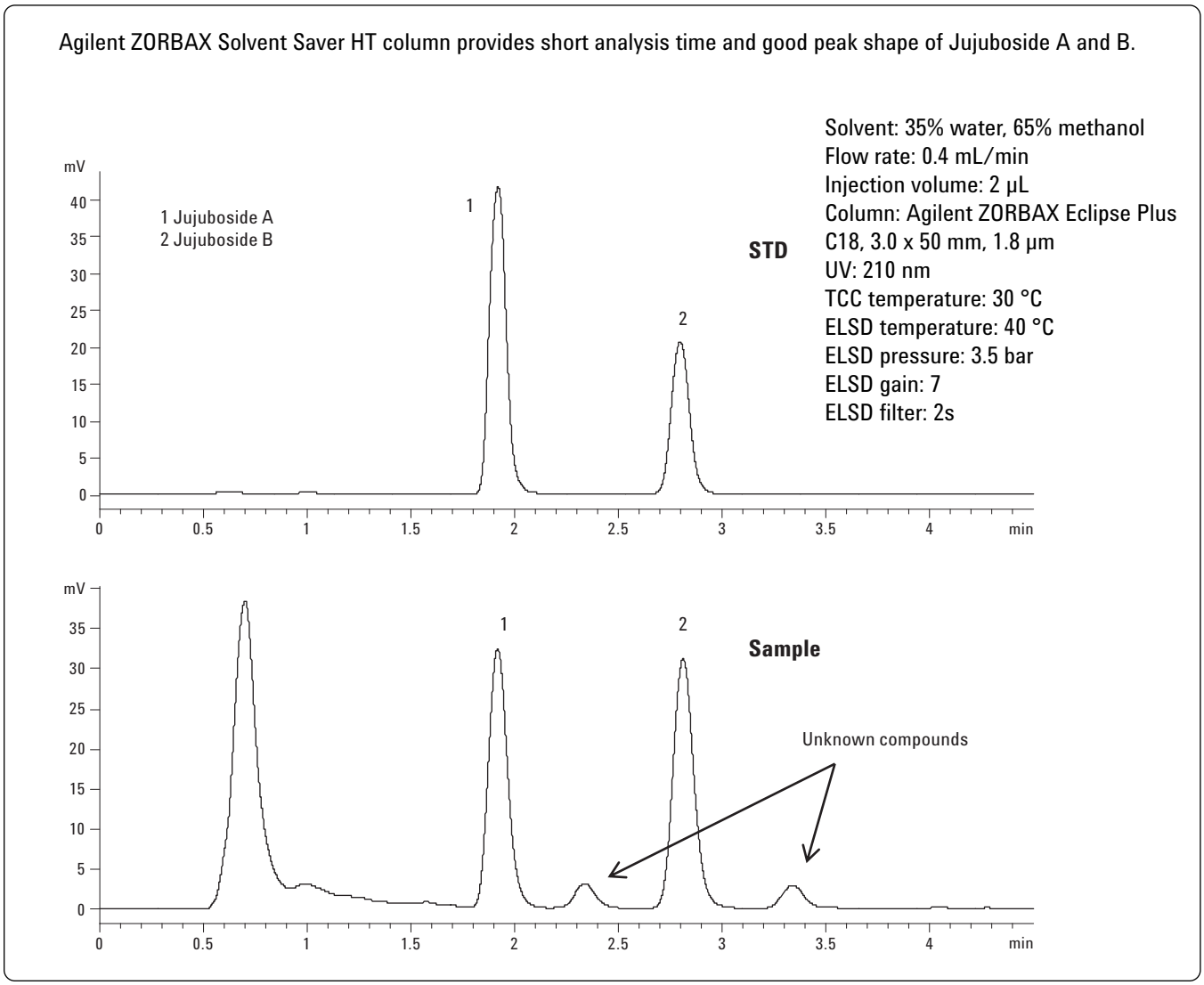


Figure 3. Standards and sample chromatograms by ELSD on Agilent ZORBAX Eclipse Plus C18, 3.0 mm × 50 mm, 1.8 µm.

Figure 4 shows the comparison of analysis time and solvent consumption between 4.6 mm × 150 mm and 3.0 mm × 50 mm columns. Changing from a 4.6 mm × 150 mm column with a 5 µm particle size to 3.0 mm × 50 mm column with a 1.8-µm particle size allows a savings of 87 percent of the solvent and 67 percent of the time. The peaks 1 and 2 show the same resolution with unknown sample compounds.

The method on 3.0 mm × 50 mm column can still be run on a standard LC instrument [3], but optimization may be required to minimize extra column volume. The ELSD detector parameters must also be optimized. When the column in the method was first switched to 3.0 mm × 50 mm, 1.8 µm column, all the parameters of the ELSD remained the same. However, the heights of the two target peaks on the 3.0 mm × 50 mm column were not consistent with the peak height obtained on the 4.6 mm × 150 mm 5 µm column. This resulted in lower theoretical plates of over two thousand for peak 1 and over four thousand for peak 2. Two parameters of the ELSD were optimized. When the sampling rate of ELSD was changed from 2.5 Hz to 30 Hz there was minimal improvement. However, when the filter value was adjusted between OFF, 2, 5 and 8, the theoretical plates increased dramatically as the filter value decreased. Baseline noise also increased simultaneously. In order to determine the ideal signal to noise value, the filter value was set to 2, which provided theoretical plates of over five thousand for peak 1 and over eight thousand for peak 2. The height of the two peaks was then consistent between the two columns, as shown in Figure 4.

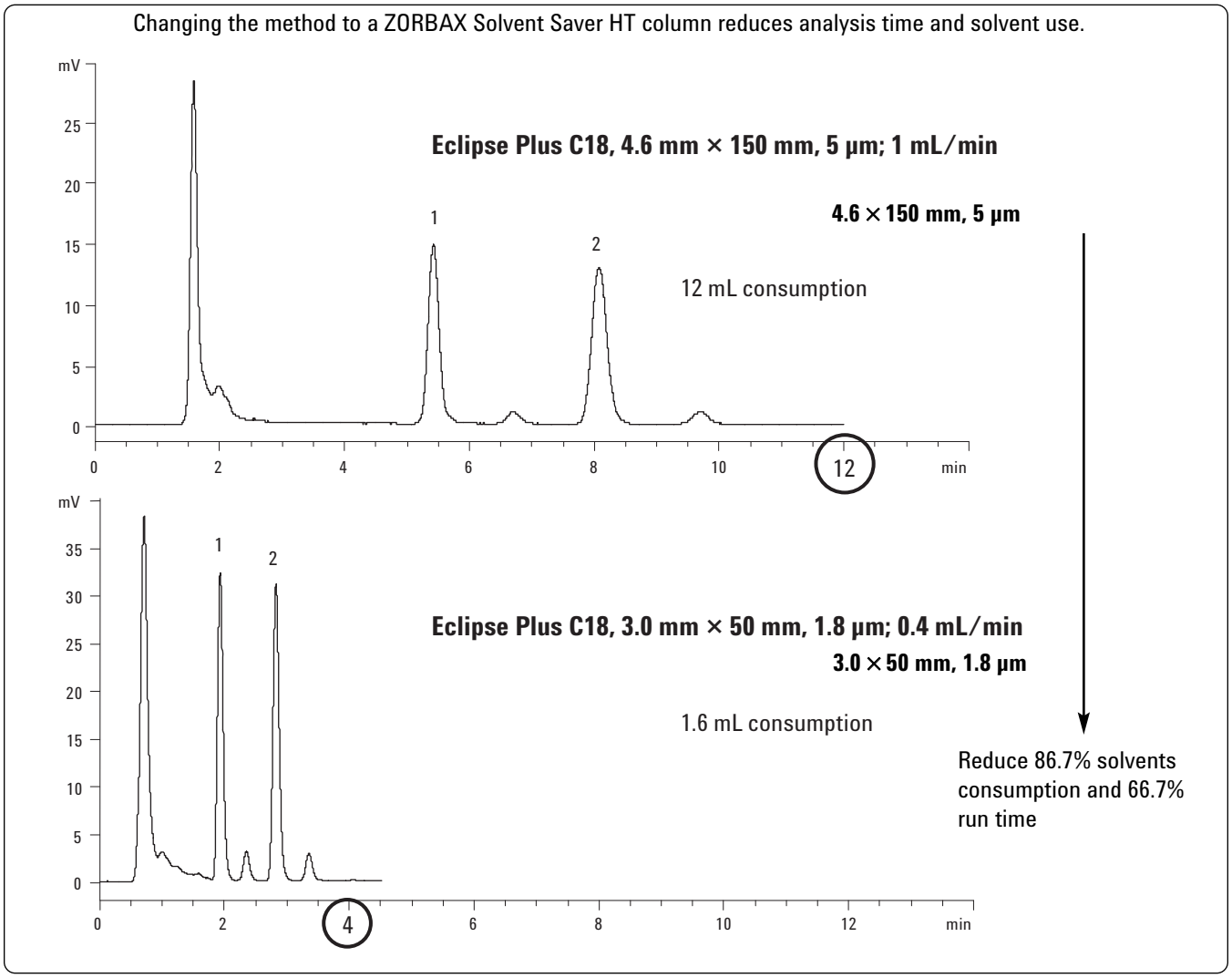


Figure 4. Comparison of the methods developed on Eclipse Plus C18 4.6 mm × 150 mm, 5 µm and Eclipse Plus C18 3.0 mm × 50 mm, 1.8 µm.

Conclusion

ZORBAX Eclipse Plus C18 can easily separate Jujoboside A and B, the two saponins of interest in spine date seed, with high efficiency and symmetrical peaks. The method developed on a 3.0 mm id Solvent Saver column dramatically reduced the solvent used and the analysis time, increasing sample throughput. An ELSD gives a cleaner baseline and higher sensitivity when separating these saponins, which makes it a better detector for these kinds of compounds in complex matrices such as traditional Chinese medicine.

Reference

1. http://www.mdidea.com/products/new/new029.html, spine date seed
2. Li Huijun and Li Ping, "Determination of Jujuboside A and Jujuboside B in Spine Date Seed by HPLC-ELSD," *Chinese Journal of Pharmaceutical Analysis*, 2000,20(2): 82-84.
3. "LC Column for Reducing Solvent Use and Waste," Agilent Technical Overview, 5990-3972EN

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Printed in the USA
June 16, 2009
5990-4176EN

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