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Rapid Analysis of Ginseng Using Accelerated Solvent Extraction and High Performance Liquid Chromatography

INTRODUCTION

Ginseng, including Asian ginseng (*Panax ginseng*) and American ginseng (*Panax quinquefolius*), are medicinal plants known worldwide. Notoginseng (*Panax notoginseng*), called Sanqi or Tianqi in China, is also a popular Chinese traditional medicine. Ginseng is believed to improve the health of people recovering from illness, improve both mental and physical performance, decrease blood glucose, control blood pressure, and impart other beneficial effects. Clinical trials in China have demonstrated the efficacy of notoginseng in promoting blood circulation, removing blood stasis (including blood clotting), relieving swelling, and alleviating pain.^{1,2}

Asian ginseng, American ginseng, and notoginseng all belong to the Araliaceae family, and contain many similar compound classes, such as ginsenosides, amyloses, fatty acids, amino acids, and polypeptides. Ginsenosides are triterpene saponins, steroid-like compounds thought to be the active components that impart the herbs' medicinal properties. Ginsenosides are classified as Ro, Ra, Rb1, Rb2, Rb3, Rc, Rd, Re, Rf, Rg1, Rg2, Rg3, Rh1, Rh2, Rh3, etc., depending on their Rf values (from small to large) by thin-layer chromatography (TLC). They can also be divided into three types based on their structures: Type A, 20(s)-protopanaxadiol (Figure 1A), Type B, 20(s)-protopanaxatriol (Figure 1B), and Type C, oleanolic acid.³

Ginseng analysis is important for manufacturers, suppliers, and researchers. Because dietary supplements

are not as strictly regulated as pharmaceuticals, the quality and contents of ginseng products and preparations can vary widely.⁴ Importers and supplement manufacturers need to be able to identify different strains of ginseng and the contents and potency of their products. Many clinical studies focus on the efficacy of one or a selected few ginsenosides. Researchers require efficient methods for extracting ginsenosides as well as methods for identifying the extracts.

Conventional extraction methods for ginsenosides, such as sonication, hot reflux, soxhlet, and immersion, are time consuming, labor intensive, and do not always deliver the desired reproducibility. Accelerated Solvent Extraction (ASE[®]) systems have the advantages of short extraction time, low solvent consumption, high extraction efficiency, excellent reproducibility, and time-saving automation. For example, conventional extraction methods for ginseng may require 2-14 h,⁵ but ASE extraction requires only 15 min. ASE instruments have been widely applied to environmental, food, and pharmaceutical extraction and analysis.⁶⁻⁸

In previously reported HPLC methods for ginsenoside analysis, either a long gradient (at least 60 min)² was needed, or few individual ginsenosides were analyzed in a single injection.⁹ This application note describes a method that combines ASE extraction with a 25 min HPLC separation for the analysis of 15 ginsenosides. This method is suitable for analyzing Asian ginseng, American ginseng, and notoginseng.

EQUIPMENT

Dionex UltiMate® HPLC system consisting of:

HPG-3400 pump

WPS-3000 Autosampler

TCC-3200 Thermostatted Column Compartment

VWD-3400 Variable Wavelength Detector

Chromeleon® Chromatography Management Software,
version 6.80

Dionex ASE 200 Accelerated Solvent Extractor

REAGENTS AND STANDARDS

Deionized (DI) water from a Milli-Q® Gradient A10 water purification system

Acetonitrile (CH₃CN), HPLC Grade (Fisher)

Methanol (CH₃OH), HPLC Grade (Fisher)

Ginsenoside standards, ginsenosides Rg1, Re, Rb1,

Rf, Rc, Rb2, Rg2, Rd, Rb3, Rh1, Rh2 and Rg3,

20(s)-potopropanaxadiol, 20(s)-protopropanaxatriol,

notoginsenoside R1 (Shanghai Pharmavan Medicine Development Co., LTD)

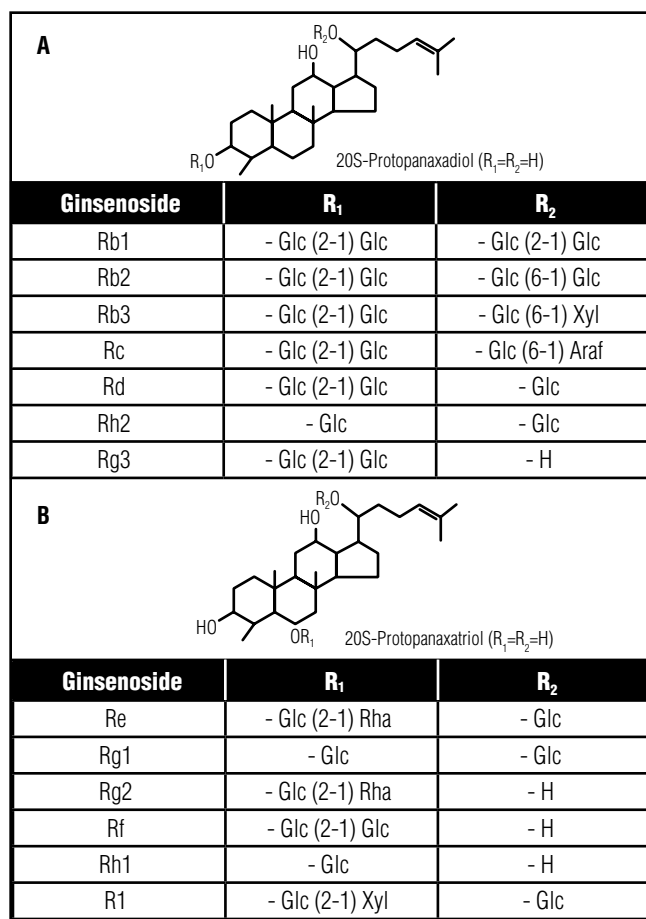


Figure 1. Structure of A) 20(s)-protopropanaxadiol, B) 20(s)-protopropanaxatriol, and 13 other ginsenosides based on these two structural skeletons.

SAMPLES

The Asian ginseng, red ginseng (a steam-cured form of Asian ginseng), and notoginseng samples used in this experiment were from China and Korea. The American ginseng samples were from America and Canada. Ginseng samples were milled to powder using a grinder or food processor.

CONDITIONS

ASE Conditions

Solvent: Methanol (100%)

Temperature: 140 °C

Pressure: 1500 psi

Static Time: 5 min

Static Cycles: 2

Flush: 20%

Purge: 100 s

Extraction

Weigh 0.5–0.7 g of each powdered ginseng sample into a separate 1-mL stainless steel extraction cell equipped with three cellulose filters on the bottom. Samples should nearly fill the cells. Extract loaded cells with the ASE system using the conditions above, and transfer the extracts into separate 25-mL volumetric flasks. Bring flasks to volume with DI water. Filter the diluted extracts through a 0.4- μ m filter prior to injection.

Chromatographic Conditions

Column: Acclaim® 120 C18, 5 μ m,
4.6 \times 250 mm, (P/N 059149)

Column Temperature: 50 °C

Inj. Volume: 2 μ L for standards and
5 μ L for samples

Mobile Phase: DI water and acetonitrile
(CH₃CN)

Flow Rate: 1 mL/min

Detection: UV at 203 nm

Gradient:

Time (min)	DI water	CH ₃ CN	Curve
0	70	30	/
10.5	60	40	5
15	0	100	5
20	0	100	5
21	70	30	5
25	70	30	5

RESULTS AND DISCUSSION

ASE Method

Optimization of ASE Method

ASE has proven effective for extracting ginsenosides from ginseng using water,¹⁰ n-butanol,² and methanol¹¹ as extraction solvents. Methanol was used as the extraction solvent in this experiment because it yielded the best extraction efficiency. ASE conditions were optimized experimentally in terms of pressure, temperature, static time, flush volume, purge time, preheating time, and cycle time. These optimized ASE conditions are shown in the ASE Conditions section. The extraction procedure required only about 15 min and about 12 mL methanol, and delivered the time and solvent savings as well as the automation typical of ASE. The ASE extract was analyzed directly, with no need for concentration or any additional purification other than simple filtration.

Efficiency and Reproducibility of the ASE Method

Each sample was extracted a second time using the optimized ASE method, and essentially no additional ginsenosides were recovered. The extraction efficiency for all the ginseng samples was > 98%. Figure 2 compares the second extraction of the Asian ginseng from Korea and a blank injection. Figure 3 shows an overlay of chromatograms for three individual extractions of notoginseng, showing good reproducibility of the optimized ASE method.

HPLC Method

Optimization of HPLC Method

Ginsenosides Re and Rg1 are two important ginsenosides with similar structure that coeluted in previous studies. Ginsenosides Rb2 and Rb3, a pair of isomers with different pharmacological effects, are also difficult to quantify because they are difficult to separate by HPLC.¹² Experiments showed that a good separation of ginsenosides Re and Rg1 could be achieved by increasing column temperature to 50 °C. Ginsenosides Rb2 and Rb3 were resolved using a simple gradient of acetonitrile. As shown in Figure 4, 15 ginsenosides, including the four ginsenosides discussed above, are separated in less than 25 min using an Acclaim 120 C18 column under the specified chromatographic conditions.

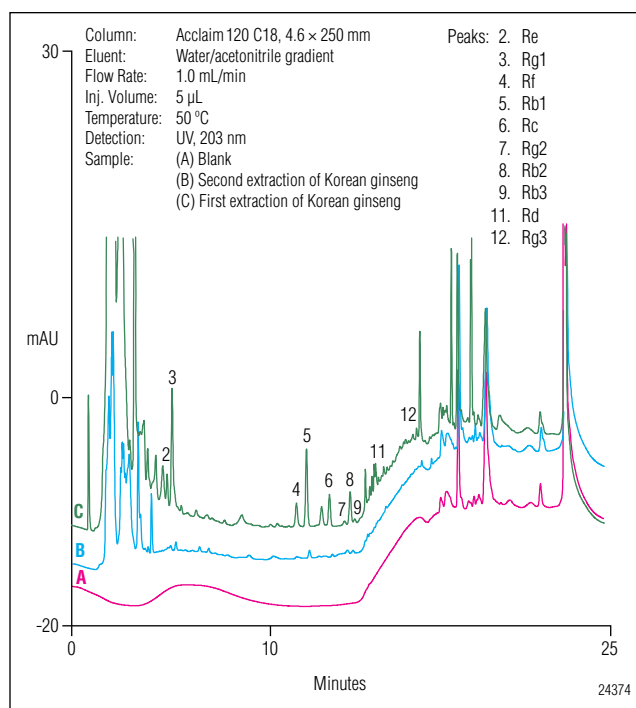


Figure 2. Overlay of chromatograms of A) a blank, B) the second extraction of Korean ginseng, and C) the first extraction of Korean ginseng. Ginsenoside peaks are nearly nonexistent in the second extract, showing that the first extraction removed essentially all of the ginsenosides.

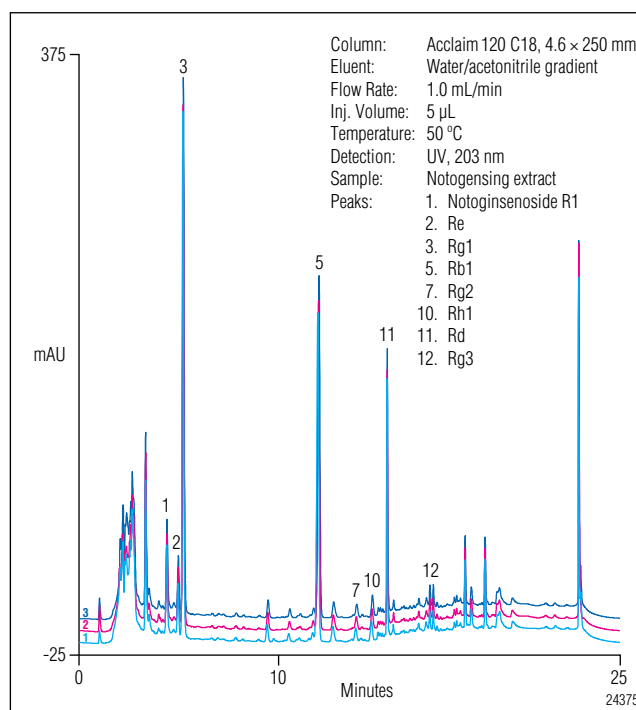


Figure 3. Overlay of chromatograms of three individual extractions of notoginseng.

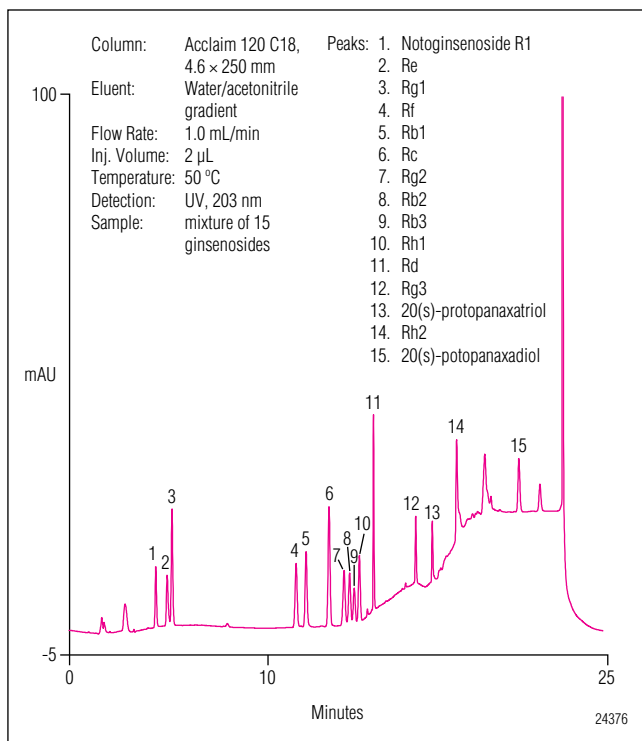


Figure 4. Chromatogram of the 15 ginsenoside standard.

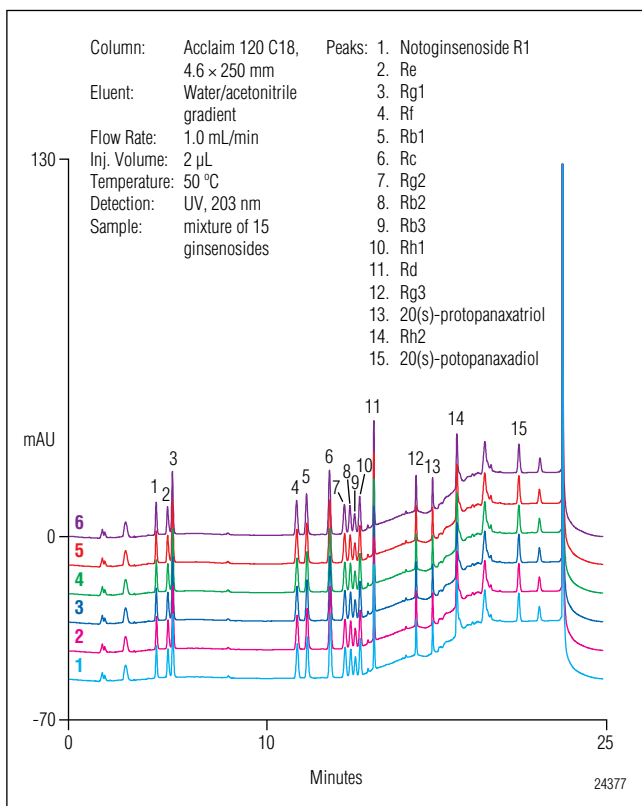


Figure 5. Overlay of chromatograms of six injections of the 15 ginsenoside standard.

Reproducibility

Figure 5 shows the reproducibility of six injections of a mixture of 15 standards; Figure 6 shows a comparison of the separation on three Acclaim 120 C18 columns. Both figures show that this 25 min separation is reproducible.

Ginseng Sample Analysis

Six ginseng samples from different species and countries of origin were analyzed (Figures 7-12). The major ginsenosides in these samples are similar, but the ginseng varieties can be identified by their characteristic saponins and the ratios of certain ginsenosides. The characteristic saponin of Asian ginseng is ginsenoside Rf, a compound not found in American ginseng. The characteristic saponin of notoginseng is notoginsenoside R1. The ratio of peak heights of ginsenosides Re and Rg1 (peaks 2 and 3) is a suitable value to differentiate the Asian ginseng (Re:Rg1 = 1:2), American ginseng (Re:Rg1 = 10:1) and notoginseng (Re:Rg1 = 1:10).¹¹

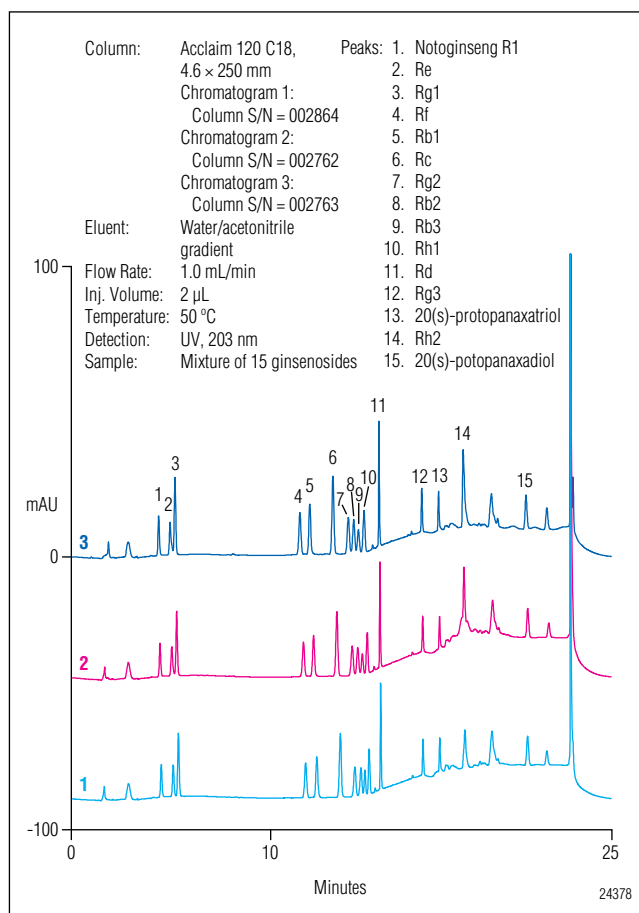


Figure 6. Chromatograms of the 15 ginsenoside standard separated on three Acclaim 120 C18 columns.

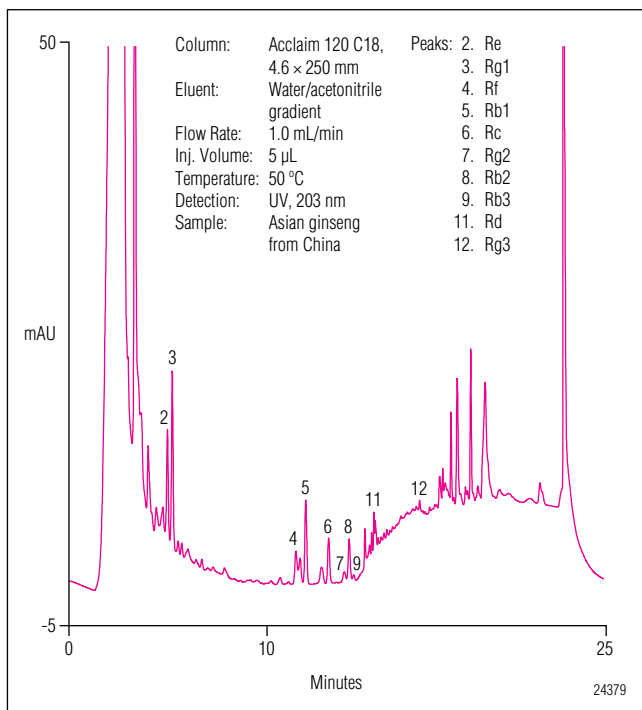


Figure 7. Chromatogram of the ASE extraction of Chinese ginseng.

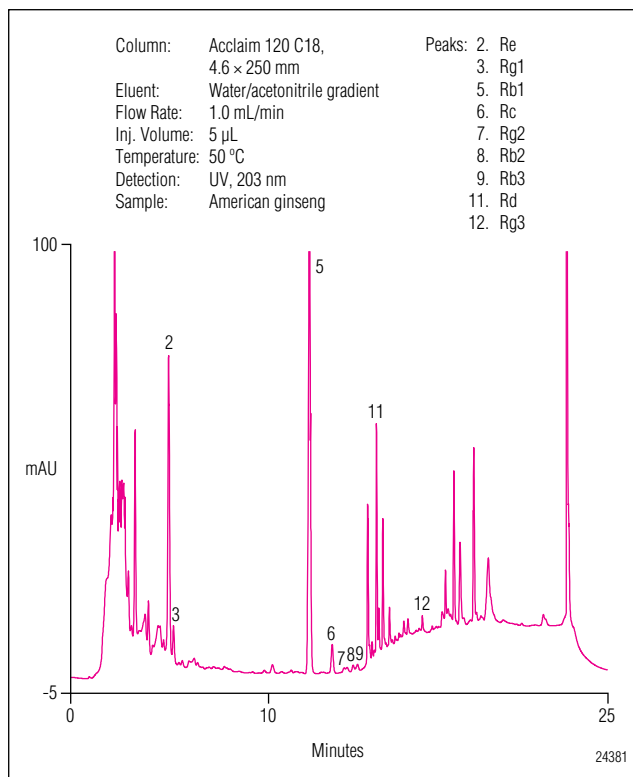


Figure 9. Chromatogram of the ASE extraction of American ginseng.

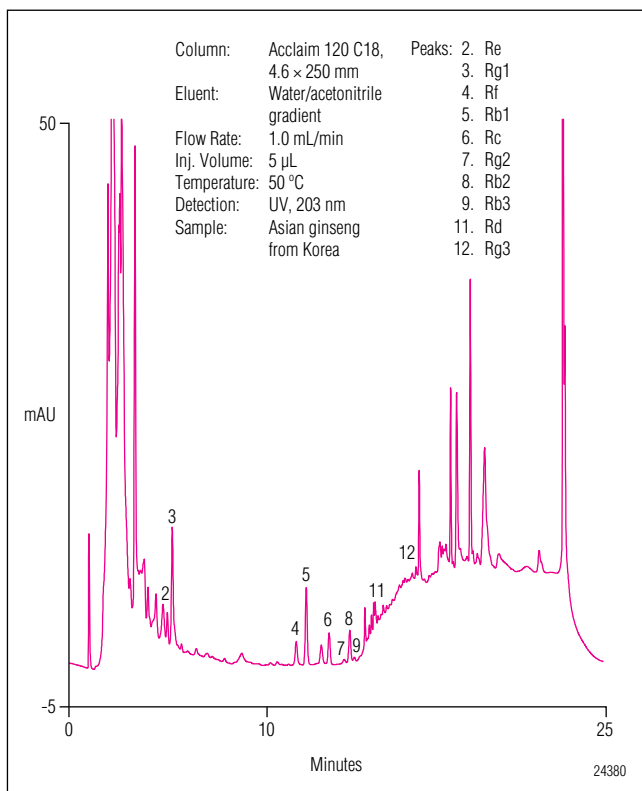


Figure 8. Chromatogram of the ASE extraction of Korean ginseng.

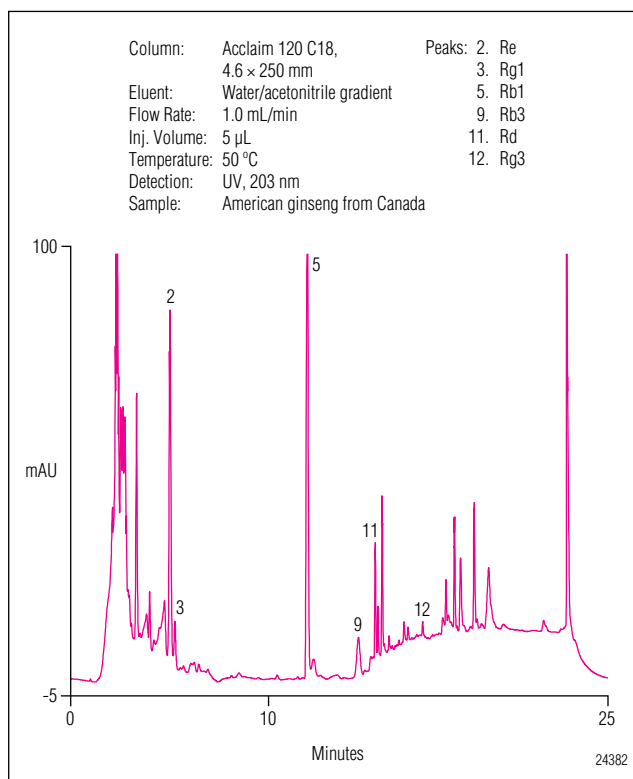


Figure 10. Chromatogram of the ASE extraction of Canadian ginseng.

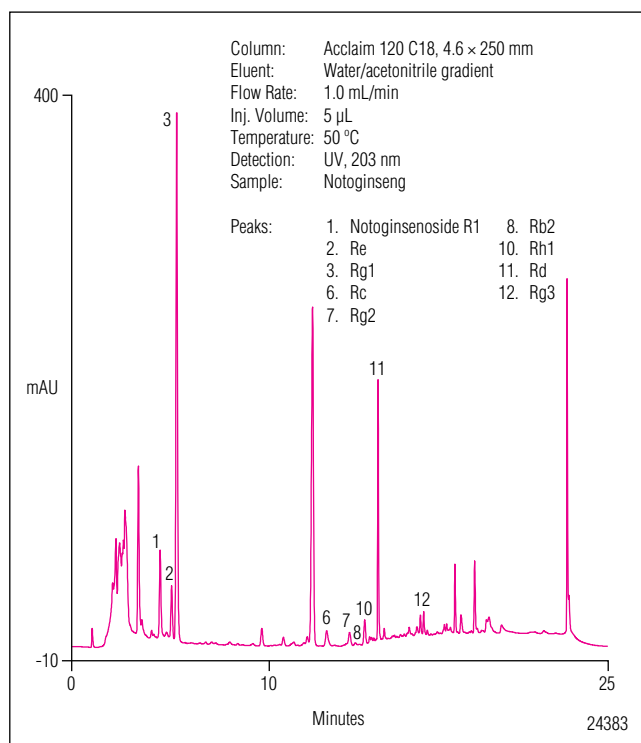


Figure 11. Chromatogram of the ASE extraction of notoginseng.

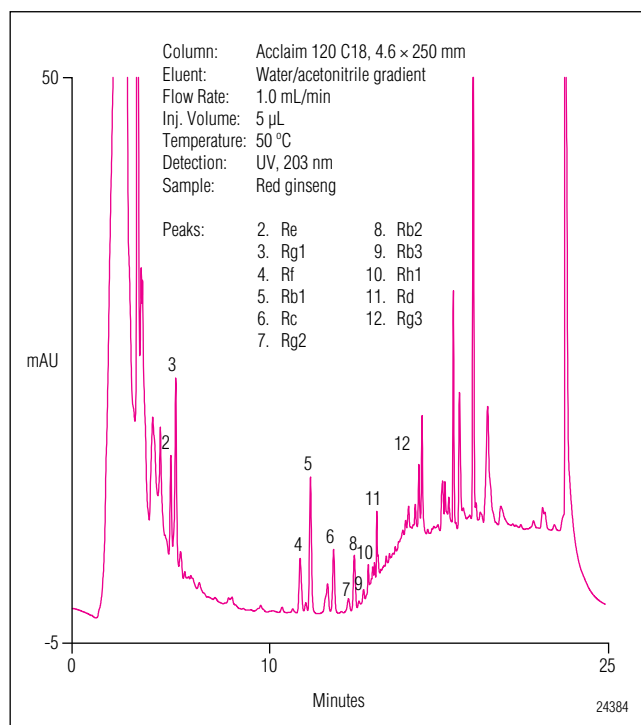


Figure 12. Chromatogram of the ASE extraction of red ginseng.

CONCLUSION

Different strains of ginseng can be identified accurately and rapidly using these extraction and analysis techniques. The use of an ASE system provides time, labor, and solvent savings as well as increased extraction efficiency and reproducibility. The rapid analysis also saves time and increases reproducibility while resolving more ginsenosides, including closely eluting peaks, in each injection.

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LPN 1965 PDF 08/07
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