DIONEX

Application Note 335

Now sold under the Thermo Scientific brand

Thermo SCIENTIFIC

Accelerated Solvent Extraction (ASE) of Active Ingredients from Natural Products

INTRODUCTION

Herbal products, nutraceuticals, and other food supplements are subject to increasingly rigorous investigation and development. Industry standardization, as well as labeling requirements under new federal regulations,[1] have prompted tighter regulation and monitoring of the active ingredients in these products. Extraction and identification of the components in raw and processed plant materials is essential for the quality control of existing products and the development of new ones. To screen the vast array of potential product candidates, laboratories must rapidly characterize raw materials for levels of active components. This is normally accomplished by extraction with liquid solvents followed by chromatographic analysis. Once products are marketed, similar analyses must be performed routinely to verify product consistency and labeling claims. While chromatographic analysis can be automated, many common extraction techniques are labor intensive, time consuming, and require large amounts of costly solvents.

Accelerated Solvent Extraction (ASE®) was developed as a solution for laboratories with increased sample throughput needs. By using common organic and aqueous solvents at elevated temperatures and pressures, ASE increases the speed and efficiency of the extraction process compared to the traditional methods. For example, sample sizes of 1–10 g are typically extracted in 12–17 min using only 10–15 mL of solvent. Solvents can be selected based on the polarity of the analyte and compatibility with any post-extraction processing steps and quantification equipment.

This Application Note describes the use of ASE for extraction of commercially available nutritional supplements, specifically hypericin from Hypericum perforatum (St. John's wort) and berberine from Hydrastis canadenis (goldenseal root). H. perforatum has been used to treat various kidney diseases, depression, and other conditions. Its therapeutic effects have been attributed to hypericin and related dianthrones (total hypericin). H. canadenis, traditionally used as an immune system booster, has relatively high concentrations of the alkaloids berberine (the most active component), hydrastine, and canadine. Hypericin, berberine, and other natural product samples can be extracted using ASE as described below.

EQUIPMENT

Dionex ASE 200 Accelerated Solvent Extractor* equipped with 11 mL cells

Dionex DX-500 HPLC System

Dionex vials for collection of extracts (40 mL, P/N 49465)

Cellulose filters (P/N 049458)

**ASE 150 and 350 can be used for equivalent results.*

REAGENTS AND STANDARDS

Acetonitrile (Optima grade; Fisher Scientific)

Water (Optima grade; Fisher Scientific)

Hypericin and berberine standards (Sigma Chemical)

Alumina (60–325 mesh, neutral; Fisher Scientific)

EXTRACTION CONDITIONS

Extraction Solvent:	Acetonitrile
Temperature:	100 °C
Pressure:	1500 psi*
Heat-up Time:	5 min
Static Time:	5 min
Flush Volume:	60%
Purge Time:	100 s
Static Cycles:	1
Total Extraction Time:	14 min per sample
Total Solvent Use:	12–15 mL per sample

**Pressure studies show that 1500 psi is the optimum extraction pressure for all ASE applications.*

SAMPLE PREPARATION

Samples were obtained from a local health food store. The materials in capsule form were opened and the ground material collected and mixed to a uniform state. Samples weighing 1–3 g were loaded into 11 mL ASE extraction cells with a cellulose filter in the cell outlet.

ANALYTICAL

Extracts of H. perforatum were adjusted to 15 mL and passed through alumina cartridges to remove co-extracted chlorophyll prior to analysis. Hypericin determination by UV-Vis absorbance at 516 nm was performed according to USP method guidelines.[2] Quantification was performed by external calibration with hypericin standard (Sigma Chemical). The value obtained is the hypericin content measured as total dianthrones. Extract volumes of H. canadenis were adjusted to 15 mL, filtered, and diluted 1:100 prior to analysis by HPLC. HPLC analysis was performed using a DX-500 HPLC (Dionex Corporation) with a 250 × 4.6 mm C8 column (Supelco, Inc). A mobile phase gradient was run with a starting ratio of 90:10 0.02 M KH_2PO_4 at pH 3.0 to acetonitrile. The ratio was adjusted to 40:60 over 10 minutes at 1.5 mL/min. UV detection at 254 nm was performed following injection of 25 µL of sample. Quantification was performed by external calibration with a berberine standard (Sigma Chemical).

RESULTS AND DISCUSSION

Hypericin

ASE extraction was performed on a commercially available St. John's wort product. Extracts were processed as described above, and examined for hypericin content by industry standard methods. The current methods used for extraction of this product are Soxhlet and sonication, both of which are time- and solvent intensive. ASE extracts were generated in 14 minutes per sample using 15 mL of solvent and were immediately ready for processing and analysis. The results for this analysis are presented in Table 1. Hypericin content (measured as total dianthrones) for this product was 0.44% with a RSD of 4.1% for the four analyses. This is consistent with a label value claiming a minimum of 0.3% hypericin.

Berberine

All ASE extractions were performed according to the method described above in "Extraction Conditions". The analysis results for this material are shown in Table 2. The average berberine content was 1.44%, with an RSD of 2.8% for the four analyses. Chromatographic profiles for the standard and extract analysis are shown in Figure 1.

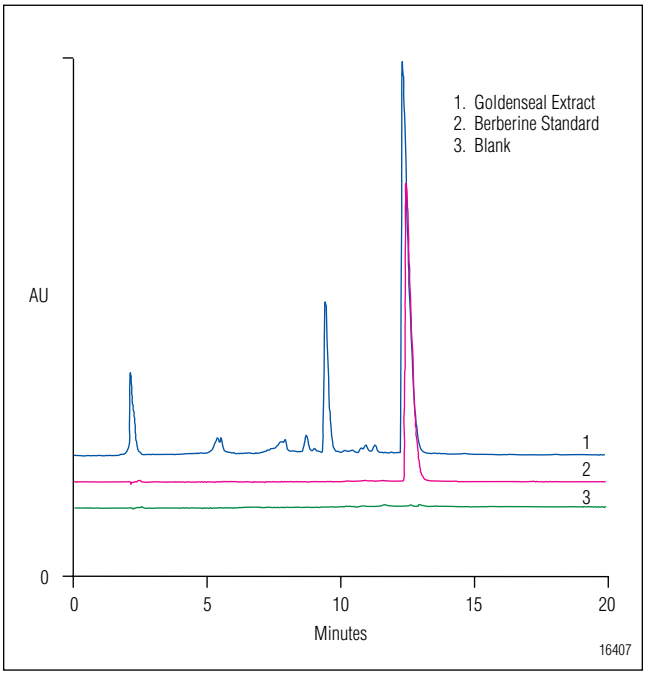

Figure 1. Chromatograms of berberine standard and goldenseal extract analysis.

In both of the applications described above, methanol was also found to be a suitable extraction solvent, and both acetonitrile and methanol are compatible with post-extraction processing steps and HPLC analysis. Water was also investigated as a potential extraction solvent. However, it also extracted a majority of the sample matrix, which complicated the determination of the active components.

Table 1. Results of ASE Extractions of *H. Perforatum*

Extract #	Hypericin Content*
1	0.43%
2	0.45%
3	0.46%
4	0.42%
Average	0.44%
SD	0.018
RSD (%)	4.1

*Measured as total dianthrones

Table 2. Results of ASE Extractions of *H. Canadenis*

Extract #	Berberine Content*
1	1.43%
2	1.43%
3	1.41%
4	1.50%
Average	1.44%
SD	0.04
RSD (%)	2.8

CONCLUSION

The extraction of natural products is essential not only as an evaluation tool for raw materials, but for the quality control of products in this growing industry. Standardization of products and labeling requirements warrant consistent measurement of active compounds in these preparations. Traditional methods of extraction can be both time consuming and labor intensive, creating delays in the flow of information from the analysis laboratory to the field or production line.

ASE was developed to increase the sample throughput of busy laboratories and deliver valuable product information. ASE works by taking advantage of enhanced solubilization kinetics that occur at temperatures higher than commonly used to perform solvent extractions. As the efficiency of the extraction process is improved, less solvent and time are required to complete the process. Results generated in many areas indicate that ASE provides extractions comparable to conventional extraction methods. However, ASE extraction is more efficient than traditional analysis in terms of time (14 min) and solvent (15 mL). In addition, ASE technology is automated, further increasing the productivity of laboratory personnel.

ASE extractions of natural products using acetonitrile or methanol can be performed rapidly. Extracted samples are immediately ready for processing or analysis by UV-Vis spectroscopy or HPLC.

REFERENCES

1. Fed. Regist. 23624, **1998**, 63.
2. Peng, T. et al. 111th Annual AOAC Intl. Meeting and Exposition; San Diego, CA, **1997**; Abstract 1102.

LIST OF SUPPLIERS

Supelco, Inc., Supelco Park, Bellefonte, PA 16823, USA. Tel: (800) 247-6628

Fisher Scientific, 711 Forbes Ave., Pittsburgh, PA 15219, USA. Tel: (412) 562-8300

Sigma Chemical, PO Box 14508, St. Louis, MO 63178, USA. Tel: (800) 325-8070

Dionex Corporation
1228 Titan Way
P.O. Box 3603
Sunnyvale, CA
94088-3603
(408) 737-0700

North America
U.S./Canada (847) 295-7500

South America
Brazil (55) 11 3731 5140

Europe
Austria (43) 1 616 51 25 Benelux (31) 20 683 9768; (32) 3 353 4294
Denmark (45) 36 36 90 90 France (33) 1 39 30 01 10 Germany (49) 6126 991 0
Ireland (353) 1 644 0064 Italy (39) 02 51 62 1267 Sweden (46) 8 473 3380
Switzerland (41) 62 205 9966 United Kingdom (44) 1276 691722

Asia Pacific
Australia (61) 2 9420 5233 China (852) 2428 3282 India (91) 22 2764 2735
Japan (81) 6 6885 1213 Korea (82) 2 2653 2580 Singapore (65) 6289 1190
Taiwan (886) 2 8751 6655

www.dionex.com

ISO 9001 REGISTERED

LPN 1115-02 PDF 4/11