Extraction of Phenolic Acids from Plant Tissue Using Accelerated Solvent Extraction (ASE)

INTRODUCTION

Polyphenols are a large family of metabolic compounds that occur naturally in a wide variety of plant foods. Recently, polyphenols have attracted a great deal of attention due to their roles as natural antioxidants. Antioxidants are believed to provide a protective effect against oxidative damage diseases such as cancer, coronary heart disease, and stroke. It has been estimated that there are approximately 8,000 naturally occurring phenolic compounds.[1] Polyphenols can be classified into two major groups: (1) phenolic acids (PA) and (2) flavonoides. Because phenolic acids exist in multiple forms, the polarity of each PA can vary significantly. This has lead to difficulty in developing a uniform extraction method for different phenolic acids from varying matrices.

ASE® is an automated extraction technique that rapidly performs solvent extractions using high temperatures and pressure. The automation of ASE allows for high sample throughput and, if needed, fast method development. ASE is accepted by several government and regulatory agencies and produces solvent extracts with similar or better recoveries than traditional extraction techniques.

This application note will focus on the extraction of phenolic acids from two different plants (eggplants[1] and black cohosh[2]) using ASE. The ASE results from five different eggplant samples are compared to eight different extraction techniques. Data from the extraction of black cohosh show the results of the optimum ASE conditions for this matrix, focusing on temperature and solvent choice.

EQUIPMENT

ASE 200 Accelerated Solvent Extractor* with solvent controller (P/N 048765)

11 mL stainless steel extraction cells (P/N 055422)

Cellulose filters (P/N 049458)

Amber collection vials, 40 mL or 60 mL (P/N 048780 or 048781)

(Note: amber vials are needed if analytes are light sensitive.)

Analytical balance (to read to nearest 0.0001 g or better)

Coffee grinder or equivalent laboratory grinder

Standard sieve (20 mesh)

ASE® Prep DE (P/N 062819)

Sand (Ottawa Standard, Fisher Scientific, Cat. No. S23-3)

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

SOLVENTS

Methanol

HPLC Water

THF (Tetrahydrofuran)

(Note: all solvents are pesticide-grade or equivalent and available from Fisher Scientific.)

SAMPLE PREPARATION AND ASE METHOD DEVELOPMENT OF PLANT SAMPLES

Sample Prep Introduction

When extracting plant samples for antioxidants, a sample size of 0.2–2 g is usually sufficient. With this small sample size, an 11-mL extraction cell or smaller will work well.

Plant material can contain a varying degree of moisture and often requires pretreatment prior to extraction. Lyophilization is an excellent pretreatment method for plant materials as this process yields a fine, dry sample that can easily be loaded into an ASE extraction cell. However, if freeze-drying is not a viable option, simply grind (or mix) the plant material with ASE Prep DE using a mortar and pestle. Initially, a ratio of plant material to DE of 1:1 is a good mixture, but the amount of DE may need to be increased if the plant material is very wet. A simple test to determine whether sufficient DE has been mixed with the sample is to observe whether the sample can be easily removed from the mortar without evidence of its adhering to the sides of the mortar. If most of the moisture has been removed, the plant material can be ground in a blender or mill to produce small particles (<2 mm). Cryo-grinders have also been used successfully.

Regardless of whether the plant sample has been pretreated to remove moisture, most samples should be mixed with a dispersing agent. The dispersing agent can simply be ASE Prep DE as mentioned above, or dry samples can be dispersed with sand. (Use 20–30-mesh particle size or Ottawa sand if available. Sea sand is not recommended as a dispersing agent and may cause premature failure of ASE instrument components.) Dispersing the sample prevents sample compaction in the extraction cell, increases the surface area, and improves solvent penetration into the sample matrix. As a result, extraction efficiency and precision improve. For dry samples, this pretreatment step should only take a few minutes, but is very important.

ASE Method Development Introduction

Optimum ASE method parameters will vary depending on the sample matrix. Each plant sample may require different temperatures and solvent mixtures.

When extracting antioxidants from plants, a good solvent mixture is methanol/water (ratios will vary with different plant samples). Also, using 100% THF has been found to work very well. When developing an ASE method for this application, choose an extraction temperature between 80 °C and 120 °C. A static cycle of 5 min is usually adequate for this application with 1 to 4 static cycles. The flush should be between 30% and 80% with a 60–120-s purge. Listed below are the ASE conditions and recoveries for the extraction of phenolic acid from two different plant matrices.

Plant Samples

Eggplant (A Black Bell variety of eggplant was obtained from a USDA farm in Riverbend, CA. Also, five cultivars of eggplant—Orient Express, Calliope Zebra Stripe, Black Beauty, Orient Charm, and Italian Neon—were purchased from a local farm.)

Black Cohosh (Freeze-dried powder of black cohosh from root and rhizome was obtained from Dr. David Lytle of the Eclectic Institute, Sandy, Oregon, USA.)

ASE CONDITIONS

Eggplant (sample size 200–500 mg)

Solvent:	CH_3OH/H_2O (80:20)
Temperature:	100 °C
Pressure:	1500 psi*
Static Time:	5 min
Static Cycles:	4
Flush:	75%
Purge:	90 s

Black Cohosh (sample size 500–1000 mg)

Solvent:	CH_3OH/H_2O (60:40)
Temperature:	90 °C
Pressure:	1500 psi*
Static Time:	5 min
Static Cycles:	2
Flush:	50%
Purge:	90 s

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

SAMPLE PREPARATION AND EXTRACTION

Eggplant

Store the eggplant samples at -60 °C under nitrogen. When ready, remove the eggplant samples, partially thaw, and peel. Chop the flesh into small pieces and freeze-dry. Grind the freeze-dried samples to a fine powder using a coffee grinder. Pass the powdered sample through a 20-mesh sieve (to a particle size of < 0.825 mm). Store the sifted powdered sample at -60°C under nitrogen until ready to extract.

Place two cellulose filters into the bottom of an 11-mL stainless steel extraction cell. Weigh approximately 200±1 mg of freeze-dried eggplant sample into the cell. Fill the remaining void volume in the cell with Ottawa sand.

Repeat this procedure for all eggplant samples and place the extraction cells on the ASE system carousel. Label and place the amber collection vials into the ASE vial carousel. Enter the ASE method listed above for eggplant and press Start.

When the extraction is complete, remove the extracts from the ASE and transfer each sample into 25-mL volumetric flasks. Adjust the total volume to 25 mL with the MeOH:H_2O solvent mixture used for the extraction. Filter appropriate aliquots of the extracts through a 0.45-μm PVDF syringe filter prior to analysis of phenolic acids by HPLC.[1]

Black Cohosh

Store the black cohosh sample at -60 °C until ready for extraction. Place two cellulose filters into the bottom of an 11-mL stainless steel extraction cell. Weigh out approximately 500–1000 mg of freeze-dried sample and mix with ASE Prep DE at a 4:1 ratio. Place the sample mixture into the extraction cell and fill the void volume with Ottawa sand. Repeat this procedure for all black cohosh samples, then place the extraction cells on the ASE system carousel. Label and place the amber collection vials into the ASE vial carousel. Enter the ASE method listed above for black cohosh and press Start.

Remove extracts and store in a dark freezer until ready for analysis. To prepare for analysis, transfer the extracts to a 25-mL volumetric flask and adjust volume to 25 mL with extraction solvent. Filter appropriate aliquots of the extracts through a 0.45-μm PVDF syringe filter prior to analysis of phenolic acids by HPLC.[2]

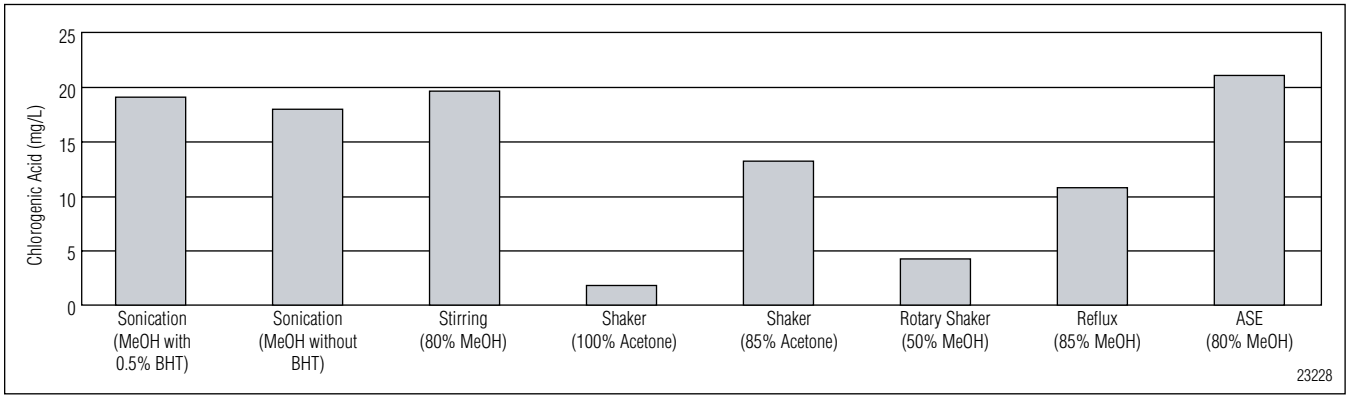

Figure 1. Comparison of extraction of chlorogenic acid from eggplant using eight different extraction methods. All samples were analyzed by HPLC.[1]

RESULTS AND DISCUSSION

Eggplant

A wide range of extraction procedures have been used for the extraction of phenolic acids from eggplant. The literature reference cited in this application note compares numerous extraction techniques to ASE.[1] Because chlorogenic acid (CA) was the predominant phenolic acid extracted from the Black Bell eggplant, the recovery of CA was used as a comparison of extraction efficiency by different procedures. Figure 1 shows the extraction efficiency of a wide variety of extraction procedures and conditions for the extraction of CA. With a solvent mixture of MeOH/H_2O at a ratio of 80:20 (v/v), ASE was found to be the optimum extraction procedure for the extraction of CA as analyzed by HPLC.[1]

Black Cohosh

The literature reference cited in this application note for the extraction of black cohosh describes optimization steps for the extraction of phenolic acids using ASE.[2] Solvent mixtures, temperature, particle size, and solvent to sample ratio were investigated.

The addition of water to the MeOH was found to increase the total phenolic (TP) recoveries. Water causes the plant tissue to swell allowing the solvent to better penetrate the sample matrix. A mixture of MeOH/H_2O at a ratio of 60:40 (v/v) produced the best results (Figure 2). Note that the use of 50% DMSO resulted in the highest total phenolic recoveries, but DMSO was not chosen as a preferred extraction solvent due to its odor and high boiling point.

Increasing the extraction temperature significantly increases analyte recoveries. The maximum TP extraction was achieved at around 90 °C. The results showed an increase by almost 30% as the temperature increased to 90 °C (Figure 3).

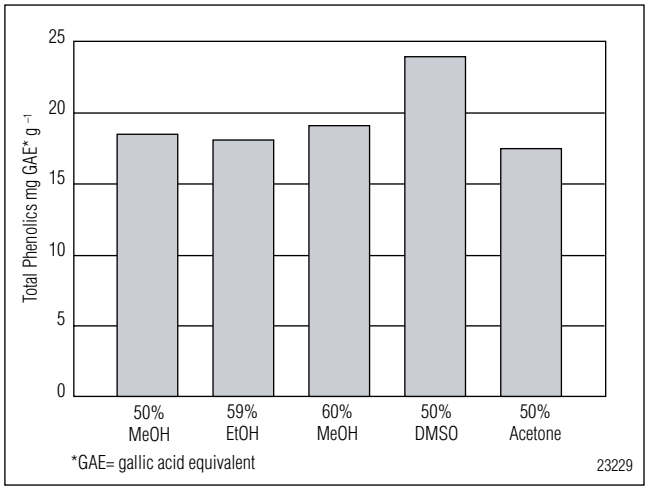

Figure 2. Comparison of various solvent mixtures on the extraction efficiency of phenolic compounds using ASE. Use of 50% DMSO resulted in the highest total phenolic recoveries. DMSO was not chosen as a preferred extraction solvent due to its odor and high boiling point. Extractions were performed at 40 ºC.

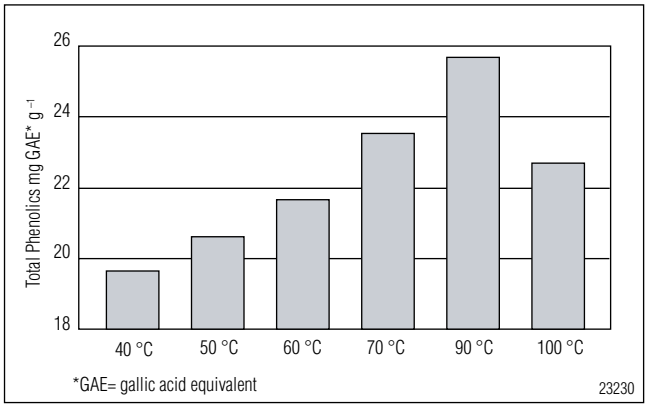

Figure 3. Influence of temperature on the extraction efficiency of phenolic compounds from black cohosh using ASE.

To achieve maximum extraction efficiency with ASE, plant samples should be finely ground. The results showed an almost threefold increase in the extraction efficiency of black cohosh as the particle size decreased from >2.00 mm to <0.25 mm. In addition, the solid-to-solvent ratio was examined by increasing the amount of sample placed into a 33-mL extraction cell while keeping the flush volumes and static cycles the same. The amount of black cohosh varied from 0.165 to 6.6 g to obtain a ratio of 5–200 mg mL^{-1}. The optimum ratio was found to be approximately 80 mg mL^{-1}.

CONCLUSIONS

ASE has been proven to be a fast, reliable extraction technology for solid and semisolid samples. This application note shows that ASE is able to extract phenolic acids from plant tissue more efficiently than traditional extraction techniques while saving time and solvent.

REFERENCES

1. Luthria, D. L. and Mukhopadhyay, S. Influence of Sample Preparation on Assay of Phenolic Acids from Eggplant. J. Agric. Food Chem. 2006, 54, 41–47.
2. Mukhopadhyay, S., Luthria, D. L., and Robbins, R. Optimization of Extraction Process for Phenolic Acids from Black Cohosh (Cimicifuga racemosa) by Pressurized Liquid Extraction. J. Sci. Food Agric. 2006, 86, 156–162.
3. Accelerated Solvent Extraction (ASE) Sample Preparation Techniques for Food and Animal Feed Samples. Technical Note 209, LPN 1781, 2006. Dionex Corporation, Sunnyvale, CA.

LIST OF MANUFACTURERS

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