

Totally Automated Sample Preparation Using Accelerated Solvent Extraction (ASE[®]) Coupled with Gilson ASPEC: The Determination of Dianthrones in St. John's Wort

INTRODUCTION

Sample preparation is one of the most time consuming and error prone portions of any analytical scheme. In spite of major advances in the instrumentation used in determinative steps, many laboratories use sample preparation techniques that are time consuming and labor intensive. 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 traditional methods. For example, sample sizes of 1 to 10 g are typically extracted in 12–17 minutes 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.

Sample extraction is only one of many steps that are utilized in laboratories that process solid samples. Filtering, volume adjustment, solid phase extraction (SPE), and solvent exchange are a few of the other procedures used. There are instruments on the market that can perform all these procedures in an automated

fashion. For example, the ASPEC is a liquid handling system from Gilson that can be programmed for the manipulation of liquid sample extracts. The coupling of the automated extraction capabilities of ASE with the automated liquid sample handling capabilities of the ASPEC system represents a major advancement in total sample preparation.

This Application Note reports on the use of a coupled ASE-ASPEC system as an automated extraction and extract manipulation device for the determination of dianthrones in St. John's Wort (*Hypericum perforatum*). The results using the coupled system are compared to those obtained using a traditional sonication procedure followed by manual SPE cleanup.

EQUIPMENT

Dionex ASE 200 Accelerated Solvent Extractor
Gilson ASPEC XL Liquid Handling System (P/N 2910815)
ASE-ASPEC Coupling Kit (P/N 2707915)
Spectrophotometer
Alumina SPE cartridges, 6-mL tubes
(P/N 57087; Supelco)

REAGENTS AND STANDARDS

Ethanol (Optima grade; Fisher Scientific)
Water (Optima grade; Fisher Scientific)
Isopropanol (Optima grade; Fisher Scientific)

SONICATION EXTRACTION CONDITIONS

Extraction Solvent: Ethanol/water (1:1, v/v)
Time: 1 h
Solvent Volume: 20 mL
Centrifuge Time: 10 min
Total Extraction Time: 70 min
Total Solvent Use: 20 mL per sample

ASE CONDITIONS

Extraction Solvent: Ethanol/water (1:1, v/v)
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

SPE CONDITIONS (MANUAL AND AUTOMATED)

Cartridge: Alumina-N, 6 mL, 2 g packing
Cartridge Conditioning: 3 mL isopropanol
Volume of Extract Used: 6 mL
Elution Solvent: Ethanol/water (1:1, v/v), 3 mL

SAMPLE PREPARATION

Samples were obtained from a local health food store. The plant material was removed from the capsules and mixed to a uniform state. Samples weighing 1 g were loaded into 11-mL ASE extraction cells that had cellulose filters placed in the cell outlets.

EXTRACT MANIPULATION

After sonication and centrifugation, the liquid was removed from the test tube containing the plant material. The final volume was adjusted to exactly 20 mL prior to SPE. For the extracts obtained using ASE, the flush volume was adjusted in the method parameters so that the volume of the extracts was 20 mL. In the manual SPE procedure, a vacuum manifold was used to process the samples, and the ASPEC used a pressure protocol to move liquids through the SPE cartridges. All volumes of solvents were the same in both manual and automated procedures as given above.

ANALYTICAL

After passing through the SPE cartridges to remove unwanted coextractable materials, the amount of dianthrone in the samples was measured at 590 nm using a spectrophotometer. The results of the sonication and manual SPE were taken as 100% because this is the standard INA method¹.

The ASPEC and ASE units were coupled using a Gilson coupling kit (P/N 2707915). The ASPEC was mounted close to the ASE system (Figure 1). A PC using 735 Sampler software (Gilson) was used to operate the ASPEC and the ASE was controlled from its front panel. The method parameters were loaded into the ASPEC along with a ready-wait command in the method. The ASPEC conditioned the SPE cartridge and then waited for the ASE to complete its extraction. When the ASE was finished, the collection vial carousel moved into position and sent a ready signal to the ASPEC.

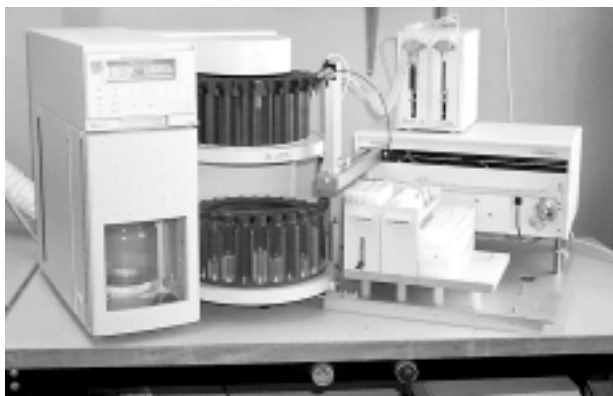


Figure 1. ASE-ASPEC coupled system.

The ASPEC then removed a 6-mL aliquot from the collection vial and loaded that onto the appropriate SPE cartridge. Next the ASPEC sent a ready signal to the ASE, allowing it to proceed with the next extraction while the ASPEC continued to process the sample using the SPE procedure. After the ASE and ASPEC completed the entire sample extraction and cleanup procedure, the samples were analyzed for dianthrone using the same spectrophotometric method as used for the manually prepared extracts. Cells filled with clean sand were used to generate blanks to zero the spectrophotometer.

RESULTS AND DISCUSSION

Table 1 compares the results of this study. Row 1 shows the data obtained using the standard sonication procedure with manual SPE prior to spectrophotometric determination of the dianthrone. Row 2 shows the data obtained using ASE as the extraction method before using the manual SPE cleanup procedure. These results are consistent with those obtained and reported previously (Dionex Application Note 335). Row 3 shows the results of coupling the ASE with the Gilson ASPEC system. As can be seen, ASE gives superior results compared to the standard sonication method. The complete automation of the procedure improves the precision of the overall process by a factor of two.

Table 1 Manual SPE After Extraction vs. Totally Automated Sample Preparation via ASE Coupled with Gilson ASPEC

	Recovery (% of Sonication)	RSD (%)
Sonication-Manual SPE	100.0	5.2 (n = 4)
ASE-Manual SPE	138.0	4.1 (n = 4)
ASE-ASPEC	135.1	1.8 (n = 8)

CONCLUSIONS

ASE was developed to increase the sample throughput and productivity of busy laboratories. ASE has proven successful in many applications and is employed routinely in widely diverse industrial uses. However, in many cases, the extraction of samples is only part of the sample preparation bottleneck. There are other sample preparation steps that are intensive manual operations that diminish laboratory productivity. The coupling of ASE with liquid handling systems such as the Gilson ASPEC can dramatically improve the overall sample throughput as well as productivity. At the same time, as shown in these results, this coupling can improve the quality of the data obtained because of the elimination of manual steps that lead to errors and poor data quality.

This Note demonstrates the use of the coupled ASE-ASPEC system for a natural product extraction, but the applicability of this system is very broad and represents a major advancement in automated sample preparation.

REFERENCES

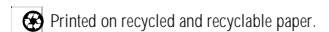
1. Institute for Nutraceutical Advancement, INA Method 104-000.

SUPPLIERS

Supelco, Inc. Supelco Park, Bellefont, PA 16823, USA.
Tel: 1-800-247-6628
Fisher Scientific, 711 Forbes Ave., Pittsburgh, PA 15219, USA. Tel: 1-412-562-8300
Gilson, 3000 W. Beltline Hwy, Box 620027, Middleton, WI 08865, USA. Tel: 1-800-445-7661



ASE is a registered trademark of Dionex Corporation.



Dionex Corporation

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

Dionex Corporation

Salt Lake City Technical Center
1515 West 2200 South, Suite A
Salt Lake City, UT
84119-1484
(801) 972-9292

Dionex U.S. Regional Offices

Sunnyvale, CA (408) 737-8522
Westmont, IL (630) 789-3660
Houston, TX (281) 847-5652
Atlanta, GA (770) 432-8100
Marlton, NJ (856) 596-0600

Dionex International Subsidiaries

Austria (01) 616 51 25 *Belgium* (015) 203800 *Canada* (905) 844-9650 *China* (852) 2428 3282 *Denmark* (45) 36 36 90 90
France 01 39 30 01 10 *Germany* (06126) 991-0 *Italy* (06) 66 51 50 52 *Japan* (06) 6885-1213 *The Netherlands* (0161) 43 43 03
Switzerland (062) 205 99 66 *United Kingdom* (01276) 691722

* Designed, developed, and manufactured under an NSAI registered ISO 9001 Quality System.



LPN 1410 6M 8/01
©2001 Dionex Corporation