White Paper 71206

# Natural Products Extraction Using Accelerated Solvent Extraction

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## Executive Summary

Accelerated Solvent Extraction is a sample preparation technique that is beneficial to laboratories extracting active ingredients from natural products. The accelerated solvent extraction technique uses elevated temperature and pressure to improve extraction efficiency and reduce extraction times to minutes per sample. Laboratories that test multiple natural product samples daily will benefit from the accelerated solvent extraction technique due to serveral key advantages over ultrasonication, shaking, and stirring including true walk-away automation and selective extractions. The Accelerated Solvent Extraction technique is an exhaustive extraction technique that ensures high recovery of target analytes in complex plant matrices and to reduces the productivity bottleneck caused by manual sample preparation techniques.

## Keywords

Dionex ASE 350 system, Dionex UltiMate 3000 system, Dionium Cells, HPLC, Acclaim Columns, Chinese Pharmacopeia, Ginseng, St. John's Wort, Black Cohosh, Goldenseal Root

## Introduction

The term natural products is a broad classification of plant compounds that includes functional foods, nutraceuticals, herbal health care, and traditional Chinese medicine. Many scientists are working to understand what compounds are present in these plant matrices that potentially cause the observed pharmacological effects and these products are subject to increasingly rigorous investigation and development. Industry standardization, as well as labeling requirements under new federal regulations,[1] has prompted tighter regulation and monitoring of the active ingredients in natural 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 released to the public, similar analyses must be performed routinely to verify product consistency and labeling claims. While the chromatographic analysis can be automated, extraction techniques such as ultrasonication, shaking, and stirring are labor intensive and time consuming. Laboratories testing natural products are required to process numerous samples that are complex in nature. Consequently, the process of sample preparation using techniques such as Soxhlet or sonication is often a severe bottleneck in the analytical workflow. Fortunately, the accelerated solvent extraction technique can be used to accelerate productivity during sample preparation.

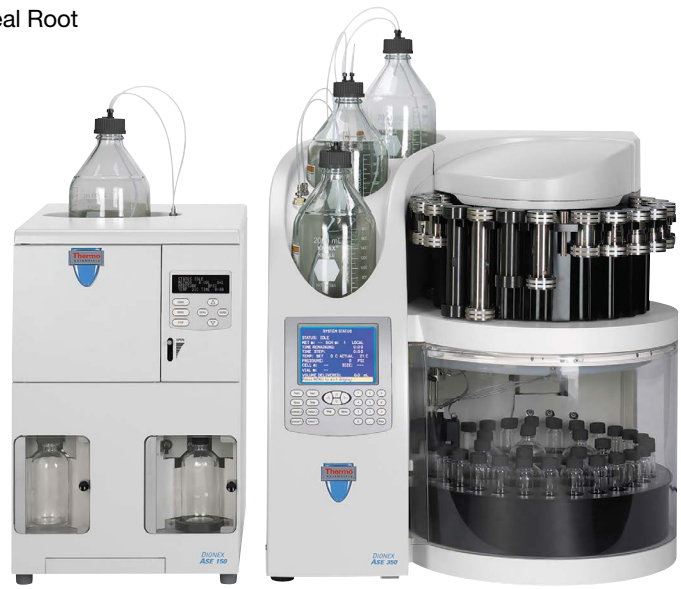

Figure 1. Dionex ASE 150/350 system.

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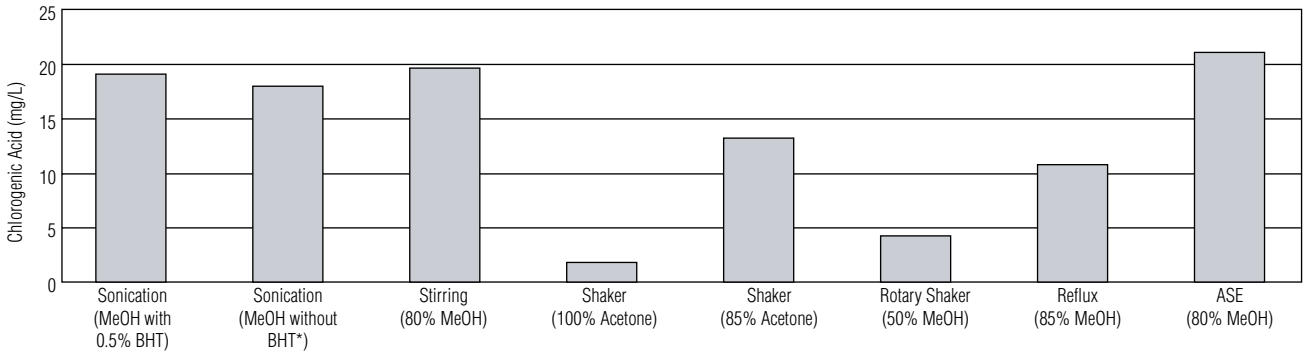
## Accelerated Solvent Extraction

Accelerated Solvent Extraction technique improves the extraction efficiency of active ingredients from natural products by using elevated temperature and pressure. Many of the organic solvents used in these extractions boil at relatively low temperatures at atmospheric pressure (e.g., acetone boils at 56 °C). By exerting a pressure of 1500 psi (10 MPa) on the solvent, the boiling point is elevated and the extraction solvent remains in a liquid state. When extractions occur at temperatures ranging from 50–200 °C, several factors contribute to improved speed and efficiency (Table 1).

Table 1. Parameters that improve extraction efficiency.

| Parameter | Effect on the Extraction Process |
|---|---|
| Temperature | Elevated temperature increases analyte diffusion from the matrix and improves analyte solubility in the extraction solvent (e.g. methanol). |
| Analyte Solubility | Increases as temperature increase to improve extraction efficiency (e.g. solubility of anthracene increases 13-fold in DCM (50–150 °C). |
| Solvent Viscosity | Decreases as temperature increases. Improves solvent migration through the matrix to increase extraction efficiency |
| Solvent Surface Tension | Decreases as temperature increases. Allows solvent to better coat the matrix and helps improve analyte diffusion. |

Figure 2 shows the results of the Accelerated Solvent Extraction technique vs. several other extraction techniques such as ultrasonication, stirring, shaking, and reflux. Chlorogenic acid was extracted from eggplant samples using eight different techniques and the extracts were analyzed using HPLC.[2] The Dionex ASE system produced the highest recovery of chlorogenic acid and thus demonstrates greater extraction efficiency than manual extraction techniques.




* BHT: Butylated Hydroxytoluene. Used as an antioxidant in accordance with sonication procedures for chlorogenic acid in eggplant.[2]

Figure 2. Comparison of extraction of chlorogenic acid from eggplant using eight different extraction methods.

## Advantages of Using Accelerated Solvent Extraction

### True Walk-Away Automation

The Thermo Scientific™ Dionex™ ASE™ 150/350 Accelerated Solvent Extractor system was designed to provide walk-away capability and can extract up to 24 samples in a single run. When using the ASE 350, an entire set of 24 samples can be set up at the beginning of the day and another set of 24 samples can be set up to run overnight with extracts that are ready for analysis first thing in the morning. The Dionex ASE system performs a sequential extraction wherein one sample is extracted at a time. This is advantagous because it ensures that each sample will see the precise conditions specified within the extraction method. Sequential extraction allows each sample, or sets of samples, to be extracted with different method conditions and permits the use of multiple sample sizes within an extraction batch. This provides flexiblity for laboratories who require automated sample preparation for numerous samples and makes the Dionex ASE system unique for automated sample preparation techniques. With minimal user intervention, 48 samples can be ready for analysis per day, providing a benefit to laboratories with high throughput needs.

### Selective Extractions

Sequential extractions are performed by the Dionex ASE system and a single sample is extracted at a time. This allows the operator to use different solvents for the extraction of multiple analytes from the same sample. For example, a plant sample can be extracted using weakly polar solvents first to remove less polar compounds of interest. Subsequently, the same sample can be extracted using a more strongly polar solvent to remove the polar compounds of interest. Each extract can be collected in a separate vial for analysis. The capability to selectively extract analytes reduces the number of coextracting compounds and reduces interference during the analysis. When using solvents with high polarity (water and methanol), the extracts are cloudy with numerous co-extracting compounds. As the polarity of the solvent is reduced, the extract becomes much more clear with few co-extracting compounds.

### Flow-Through Design

The Dionex ASE extraction cells are designed for a solvent to enter the top of the cell and exit at the bottom with the extracted analytes. This design permits the analyst to add sorbents to the bottom of the cell in order to remove unwanted co-extractables during the extraction process. This helps eliminate post extraction clean up steps and decrease the amount of time spent preparing samples for analysis. Since many natural product samples must be cleaned or filtered, the use of the Dionex ASE system eliminates this labor-intensive step and improves productivity prior to analysis.[3,4]

### Dynamic and Static Extractions

Dynamic and static extraction cycles can be performed in the same run by the Dionex ASE system. Dynamic extraction is defined as the ability to introduce fresh solvent during the extraction process. This is important because it ensures that the extraction solvent will not become oversaturated with the analyte, decreasing its ability to remove more analyte from the matrix. Static extraction is defined as holding the extraction solvent and sample for a set period of time to maximize the solubility of the analytes. Performing both dynamic and static extractions allows the Dionex ASE system to be an exhausitve extraction technique and ensures maximum analyte recoveries. This functionality ensures that the quantity of active ingredients present in each natural product sample are determined with a high degree of accuracy. Table 2 shows the results of a comparative study for the ASE, Soxhlet, and sonication for extracting terpenes, fatty acids, and Vitamin E from *Piper gaudichaudianum Kunth*.[3] The ASE was able to produce higher recoveries of the target compound per kg of sample in comparison to Soxlhet and sonication.

Table 2. Comparative study for Soxhlet, ASE, and sonication.

| | Soxhlet | | Sonication | | ASE | |
|---|---|---|---|---|---|---|
| | Yield* | SD%** | Yield | SD% | Yield | SD% |
| Nerolidol | 3435.63 | 1.79 | 271.18 | 2.01 | 2144.51 | 2.90 |
| Palmitic acid | 2853.91 | 0.85 | 1359.24 | 1.50 | 3793.93 | 3.10 |
| Phytol | 254.08 | 1.69 | 82 | 0.41 | 309.14 | 0.98 |
| Stearic acid | 956.56 | 0.15 | 767.45 | 1.52 | 1234.54 | 1.37 |
| Squalene | 80.16 | 0.05 | 4.45 | 0.02 | 98.12 | 1.89 |
| Vitamin E | 432.31 | 0.14 | 68.84 | 0.16 | 576.04 | 2.49 |
| Stigmasterol | 963.98 | 0.25 | 219.43 | 1.07 | 1205.31 | 3.01 |
| Beta-Sitosterol | 106.92 | 0.62 | 46.51 | 0.04 | 194.28 | 1.89 |

* Concentration (mg of the compound/kg of sample)

** Percent standard deviation (n = 3)

## Application Notes Summary

### AB 102: Determination of Aucubin, Genipoide, and Pinoresinal Diglucoside in Cortex eucommiae Using ASE and HPLC

This application brief describes an efficient method for the determination of aucubin, genipoide, and pinoresinal diglucoside in *Cortex eucommiae*, herbs commonly used in Chinese medicine as tissue restoratives, using accelerated solvent extraction technique and the Thermo Scientific™ Dionex™ UltiMate™ 3000 HPLC system. The use of the accelerated solvent extraction technique for the extraction of C. *eucommiae* replaced the Soxhlet extraction method in the Chinese Pharmacopeia Edition.[6] The Soxhlet method required 500 mL of two organic solvents and an extraction time of twelve hours per sample. The accelerated solvent extraction technique reduces the solvent use to appoximatley 30 mL per sample required only 15 min of extraction time for each sample. Using a Thermo Scientific™ Acclaim™ PolarAdvantage (PA) column and UV detection, the three active componets in Cortex eucommiae are resolved in less than 15 min.

### AN 192: Rapid Analysis of Ginseng Using Accelerated Solvent Extraction and HPLC

Ginesonosides are used in both traditional Chinese medicine as well as over the counter nutraceutial pills in North America and Europe. Importers and supplement manufactuers need to be able to identify different strains of ginseng and the contents and potency of their products. The limitation in this analysis has historically been the extraction of ginsenosides from the matrix using techniques such as ultrasonication, Soxhlet, and immersion. By using accelerated sovlent extraction, the method presented in this application requires approximately 30 mL of solvent and 15 min of extraction time per sample. Using an Acclaim 120 C18 column and UV detection, all 15 gineosonoside compounds are resolved in 25 min. This method is suitable for analyzing Asian ginseng, American ginseng, and notoginseng.

### AN 207: Chromatographic Fingerprinting of *Flos chrysanthema* Indici Using HPLC

The plant *Flos chrysanthemi* is a common medicinal plant known in China as wild chrysanthemum. The Chinese Pharmacopeia Edition regulates its use as an herbal medicine.[6] This plant is used with the belief that it improves eyesight and cures fever, swelling, erysipelas (a bacterial infection of the skin), sore throat, and headache. This application note establishes an HPLC chromatographic fingerprint of F. *chrysanthema* based on discriminating the characteristic peaks of chlorogenic acid and flavonoids (luteolin-7-o-glucoiside, linarin, lueteolin, and apigenin) using a Dionex UltiMate 3000 HPLC. Using an Acclaim C18 column and UV detection, 22 homo-chlorogenic acids and flavonoids were resolved in 50 min to provide a chromatographic fingerpint. An ultrasonic extraction technique was compared to the accelerated solvent extraction technique for isolation of the target components from the samples. Both sample preparation techniques were able to extract the same major compounds but the accelerated solvent extraction technique demonstrated higher extraction efficiency for the compounds eluting before 25 min.

### AN 232: Determination of Anthraquinones and Stilbenes in Giant Knotweed Rhizome by HPLC with UV Detection

Giant knotweed rhizome, the dried rhizome and root of *Polygonum cuspidatum* is a common medicinal plant in China and the Chinese Pharmacopeia regulates its use as an herbal medicine.[6] It is used with the belief that it cures angiocardiopathy, skin inflammations, liver diseases, reduces fever, and relieves arthritis pain. This application note describes an HPLC method that determines the eight main active components from giant knotweed rhizome in a single injection (anthraglycoside A, anthraglycoside B, emodin, physcion, rhein, chrysophanol, resveratrol, and polydatin). Using the UltiMate 3000 system with an Acclaim 120 C18 column and UV detection, eight active components in giant knotweed rhizome were resolved in 25 minutes. The Dionex ASE system was used for the extraction and demonstrates that it can be used for complex samples such as the giant knotweed.

### AN 335: Accelerated Solvent Extraction (ASE) of Active Ingredients from Natural Products

This application note describes the use of accelerated solvent extraction technique for extraction of commercially available nutritional supplements; specifically hypericin from *Hypericum perforatum* (St. John's Wort) and berberine from *Hydrastasis canadenis* (Goldenseal root). The current methods used for extraction of these products are Soxhlet and sonication. In this application, accelerated solvent extraction technique generated in 14 min per sample using approximatley 40 mL of solvent and did not require offline clean up prior to analysis.

### AN 357: Extraction of Phenolic Acids from Plant Tissue Using Accelerated Solvent Extraction

The extraction of phenolic acids from two different plants (eggplants and black cohosh) using accelerated solvent extraction technique is the focus of this application note. The use of accelerated solvent extraction technique was compared to ultrasonication, stirring, shaking, and reflux for sample preparation. The accelerated solvent extraction technique was able to generate 20 mg/L vs. 3 mg/L with sonication among these techniques, demonstrating an advantage for automated sample preparation and laboratories looking to decrease the amount of analyst intervetion in the extraction process.

### AN 362: Extractions of Herbal Market Compounds Using Accelerated Solvent Extraction Compared to Traditonal Pharmacopoeia Protocols

The use of the Dionex ASE system for solvent extraction of five marker compounds (atropine, caffeine, boldine, casticine, and parthenolide) from herbal preparations followed by analysis by HPLC is reported in this application note. Researchers at the University of Basel in Switzerland compared the percent recovery of these five compounds to the extraction results from traditional pharmacopeia techniques. Extraction results for each compound exceeded 100% when using the accelerated solvent extraction technique (listed as percent recovery relative to the traditional extraction techniques described in the Swiss pharmacopoeia monographs), demonstrating that the accelerated solvent extraction technique is more efficient that Soxhlet or ultrasonication techniques.[7]

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