DIONEX

Application Note 362

Extraction of Herbal Marker Compounds Using Accelerated Solvent Extraction Compared to Traditional Pharmacopoeia Protocols

INTRODUCTION

The extraction step in quantifying marker compounds in herbs is critical to achieving meaningful dosage data. Laboratories typically spend more time focusing on validating the methods used for sample analysis than developing and validating an extraction method.[1] Sample preparation can be the most important step in the development of analytical methods for the analysis of constituents present in botanicals and herbal preparations.[2] The Accelerated Solvent Extraction system (ASE®) uses elevated temperature and pressure to increase the efficiency of the extraction process.[3] ASE automates and improves solvent extraction by significantly decreasing solvent consumption and extraction time. This application note describes the use of the ASE system for solvent extraction of five marker compounds from herbal preparations followed by analysis by HPLC. The results are compared to traditional pharmacopoeia extraction methods.[4]

EQUIPMENT

Dionex ASE 350 Accelerated Extractor (P/N 066050)
Extraction cells, 10 mL stainless steel (P/N 068093)
Cellulose Filters (P/N 049458)
Collection Vials, 60 mL (P/N 048781)
Standard Laboratory Sample Evaporation System
Standard Laboratory Grinder or Mill

CHEMICALS AND REAGENTS

Dichloromethane (DCM) (HPLC grade, Fisher Scientific)
Methanol (CH_3OH) (HPLC grade, Fisher Scientific)
Ammonia Hydroxide (NH_4OH) (Fisher Scientific)

SAMPLES

Table 1. Samples and Analytes

Plant Material	Herbal Marker Compound
Atropa belladonna L. (Belladonna leaf)	Atropine
Cola nitida (Cola seed)	Caffeine
Peumus boldus Mol. (Boldo leaf)	Boldine
Vitex agnuscastus L. (Chaste tree fruit)	Casticine
Tanacetum parthenium L. (Feverfew leaf)	Parthenolide

Plant samples were purchased from Dixa AG (St. Gallen, Switzerland)

Table 2. ASE Conditions					
Sample	**Belladonna Leaf**	**Cola Seed**	**Boldo Leaf**	**Chaste Tree Fruit**	**Feverfew Leaf**
Amount (sieve size, µm)	1 g (180)	1 g (355)	1 g (355)	1 g (355)	1 g (355)
Static Cycles	3	2	3	2	1
Solvent	DCM + 2 mL Ammonia Solution*	CH_3OH (100%)	DCM + 2 mL Ammonia Solution*	CH_3OH (100%)	CH_3OH (100%)
Preheat**	1 min	1 min	1 min	1 min	1 min
Pressure	120 bar (~1500 psi)	120 bar (~1500 psi)	120 bar (~1500 psi)	120 bar (~1500 psi)	120 bar (~1500 psi)
Temperature	70 °C	70 °C	70 °C	70 °C	70 °C
Static Time	5 min	5 min	5 min	5 min	5 min
Flush	100%	100%	100%	100%	100%
Purge	120 sec	120 sec	120 sec	120 sec	120 sec

**Ammonia solution (NH_4OH) = 33% (m/v)*

***Preheat step is not recommended for extraction of volatile analytes*

SAMPLE PREPARATION

Prior to extraction, grind the samples to less than 1 mm in size. Weigh 1 g of each sample type and add to a 10 mL extraction cell containing a cellulose filter. (Moisten the Belladonna and Boldo leaf samples with 2 mL of the ammonia solution (33% m/v) in the extraction cell prior to extraction.)

EXTRACTION

Place the extraction cells containing the pre-weighed sample onto the ASE extraction cell carousel. Place the appropriate number of collection vials into the collection vial carousel. Create Methods for each sample as described in Table (Table 2). Enter these Methods into a sequence and save. Load this sequence and press "Start" on the front panel to begin the extraction sequence.

RESULTS

Analysis of the extracts was performed using HPLC.[4] Results for each sample are listed in Table 3 with recoveries listed as percent recovery relative to the traditional extraction techniques described in the Swiss pharmacopoeia monographs.

Table 3. Results of ASE Extraction Compared to Traditional Swiss Pharmacopoeia Methods		
Plant Material	**Herbal Marker Compound**	**Recoveries**
Atropa belladonna L. (Belladonna leaf)	Atropine	147.0%
Cola nitida (Cola seed)	Caffeine	100.5%
Peumus boldus Mol. (Boldo leaf)	Boldine	343.0%
Vitex agnuscastus L. (Chaste tree fruit)	Casticine	142.9%
Tanacetum parthenium L. (Feverfew leaf)	Parthenolide	108.2%

CONCLUSIONS

ASE systems provide a more efficient extraction method for herbal marker compounds than traditional methods. The combination of elevated temperature and pressure provides more efficient and economical solvent extraction by saving time and solvent compared to the methods described in the Swiss pharmacopoeia.

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REFERENCES

1. Raynie, D.E. Modern Extraction Techniques. *Anal. Chem.* **2006,** *78*: 3997–4003.
2. Ong, E.S. Extraction Methods and Chemical Standardization of Botanicals and Herbal Preparations. *Journal of Chromatography B* **2004,** *812 (1–2)*: 23–33.
3. Richter, B.E.; Jones, B.A.; Ezzell, J.L.; Porter, N.L.; Avdalovic N.; Pohl, C. Accelerated Solvent Extraction: A Technique for Sample Preparation. *Anal. Chem.* **1996,** *68*: 1033–1099.
4. Basalo, C., Mohn, T., and Hamburger, M. Are Extraction Methods in Quantitative Assays of Pharmacopoeia Monographs Exhaustive? A Comparison with Pressurized Liquid Extraction. *Planta Med.* **2006**, *72(12)*: 1157–1162.

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