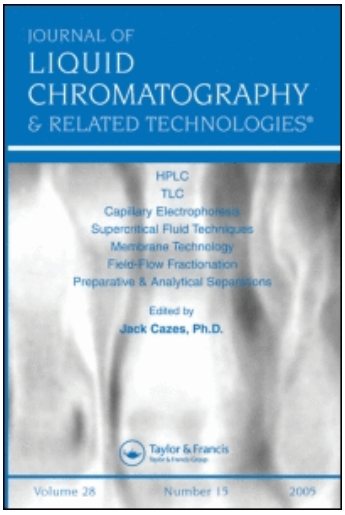

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PRESSURIZED LIQUID EXTRACTION AND MULTIPLE, ULTRASONICALLY-ASSISTED EXTRACTIONS OF HYDRASTINE AND BERBERINE FROM GOLDENSEAL (*HYDRASTIS CANADENSIS*) WITH SUBSEQUENT HPLC ASSAY

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ABSTRACT

Procedures involving either multiple ultrasonically-assisted extractions or pressurized liquid extractions for extracting hydrastine and berberine from goldenseal (*Hydrastis canadensis*) prior to assay by HPLC are presented. Both procedures provide significant time advantages over Soxhlet extraction, while yielding commensurate results. The laboratory-assembled pressurized liquid extraction apparatus is described and used to facilitate the assay of hydrastine and berberine in some goldenseal products.

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INTRODUCTION

Goldenseal (*Hydrastis canadensis*) is an herb that is well known for its high content of isoquinoline alkaloids of which berberine is the most studied. The plant has shown antibiotic, immunostimulating, anticonvulsant, and other pharmacological activity.(1) To enable manufacturers to provide a consistent, high-quality product of known potency, the USP has provided an HPLC assay for the alkaloids hydrastine and berberine and delineated lower limits of 2.0% for the former and 2.5% for the latter in root powder.(2) Unfortunately, the sample handling requires a minimum of 6 hours continuous (Soxhlet) extraction with methanol, resulting in a serious bottleneck for routine analysis. Other workers also specify laborious extraction procedures prior to HPLC(3) and CE.(4)

The purpose of this work was to investigate reducing the time for exhaustive extraction of the alkaloids from goldenseal. Initially, the efficacy of simple ultrasonically-assisted extractions was examined. Pressurized liquid extraction was also examined. This technique, also called accelerated solvent extraction, and consisting basically of pressurized extraction with superheated liquids, was developed to overcome matrix-dependent problems associated with supercritical-fluid extraction.(5) Although it was initially developed to replace Soxhlet extraction in environmental analysis, this technique has recently been applied to medicinal plants with an automated commercial apparatus.(6) A simple laboratory-assembled pressurized liquid-extraction apparatus was used to extract berberine from *Coptidis rhizoma*.(7) A somewhat different laboratory-assembled apparatus was investigated here for extraction of the goldenseal alkaloids. Results were compared with that obtained with ultrasonically-assisted and Soxhlet extractions.

EXPERIMENTAL

Reagents and Chemicals

Berberine chloride and hydrastine were obtained from Sigma. Purity was estimated by HPLC area % at 235 nm and was found to be 98.5% or better. Water in berberine chloride was determined to be 10.84% by loss on drying. All results using the berberine chloride standard are presented as berberine. The following goldenseal-containing products and raw materials were purchased from local retailers or from mail order, and are prefixed by sample identification numbers in half-parenthesis, followed by lot numbers in full parenthesis:

1) Nature's Resource Golden Seal Root (707251), Nature's Resource Products, Mission Hills, CA;

2) Nature's Resource Standardized Goldenseal (IL13094);

3) Herbal Classics Goldenseal (E989), Vitamin Classics, Inc., Calabasas, CA;

4) CVS Standardized Goldenseal (8E00817), CVS, Woonsocket, RI;

5) Nature's Way Goldenseal Root, 5% Total Alkaloids (905218), Nature's Way Products Inc., Springville, UT;

6) Spring Valley Golden Seal Root (905041), NaturPharma, American Fork, UT;

7) Scientific Herbals Goldenseal Root Powder (GGH9901A), Graham Development, Inc., Oneonta, NY;

8) Scientific Herbals Standardized Goldenseal (GG0001A);

9) Goldenseal Root Powder (46595-51), Starwest Botanicals Inc., Rancho Cardova, CA.

Product 9 root powder was used for method development. Product 4 was assayed 9 months past the expiration date. None of the other products were assayed beyond the posted expiration date. All other reagents were HPLC or ACS quality, and were used without further purification.

Apparatus

For part of a solvent study, a Bellco Sci Era Hot Shaker water bath set at 50°C was used. The pressurized liquid extraction apparatus was assembled from an ISCO Model 260 D syringe pump with a Series D Pump Controller, a Keystone Scientific 9.7-mL stainless steel supercritical-fluid extraction cell housed in a Cole Parmer Model 05015-50 laboratory oven, three high-pressure two-way shut-off valves, a splitting T, and an on-off valve connected to a nitrogen tank. All connections were made with 1/16" stainless-steel HPLC tubing and fittings. The cell contained a stainless-steel frit on both the inlet and outlet ends.

The apparatus, shown in Figure 1, is basically a supercritical-fluid extraction apparatus with the restrictor replaced by a shut-off valve. It differs from commercial automated multicell instruments (e.g., Dionex, Applied Separations, ISCO) and the laboratory-assembled instrument described by Ong and coworkers,(7) in that the pressure is maintained by the syringe pump operated in a constant pressure mode. Pressure is maintained in the commercial instruments by a static valve that opens when the pressure exceeds a preset amount, while in the other laboratory-assembled apparatus, it is controlled by material arbitrarily packed in the cell and tubing system.(7) The latter could only be operated dynamically. That laboratory-assembled instrument, and the one assembled here, was manually operated.

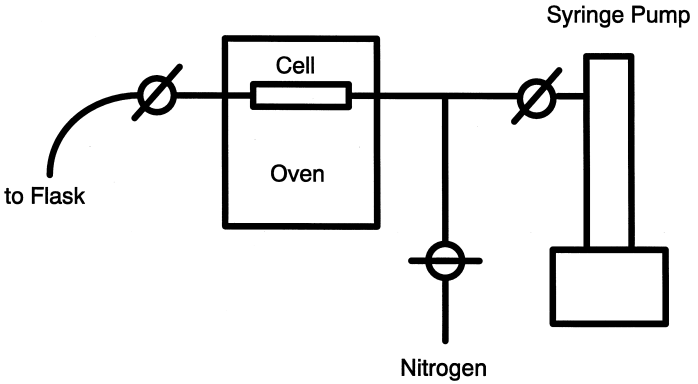

Figure 1. Pressurized liquid extraction apparatus. The shut-off valve used in refilling the syringe pump is not shown.

HPLC System

The HPLC used was a Waters Model 2690 Separations Module with a Model 996 Photodiode Array Detector. The system was operated with the Millenium32 Chromatograph Manager version 3.00 software. The HPLC operating parameters are presented in the USP.(2) The column was a 150 x 4.6-mm, 5 µm Phenomenex Luna C_{18}, pumped isocratically with an acetonitrile:aqueous 0.1 M monobasic potassium phosphate (27:73) eluent monitored at 235 nm. Because of pressure considerations, the flow rate was lowered from the 1.8 mL/min USP value to 1.2 mL/min resulting in an increase in run time from 10 to 15 minutes. The injection volume was also decreased from 10 to 5 µL.

Standard Preparation

Three standard solutions, ranging in concentration from 130-800 µg/mL hydrastine and 100-640 µg/mL berberine, were prepared by serial dilution of the respective 2000 µg/mL and 1600 µg/mL stock solutions with methanol:water (70:30). Typical linearity data for hydrastine and berberine determined over the above range by plotting peak area at 235 nm *versus* µg/mL are presented in Table 1.

Concentrations were corrected for HPLC purity, water determined by LOD, and molecular weight considerations. Standard solutions were stored in a freezer at –20°C when not in use, and were found to yield constant response and consistent results for at least 8 months.

Table 1. Linearity

Parameter	Hydrastine	Berberine
Correlation coefficient	0.999996	0.999997
Slope, area units mL/μg	7632	17639
Intercept, area units	−2788	−10860
Standard error of the estimate ($S_{y/x}$), area units	10606	16249
% Intercept[a]	−0.1	−0.2
% Variation[b]	0.3	0.3

[a](y intercept/$\bar{y}$) × 100, where $\bar{y}$ is the average y[9].
[b]($S_{y/x}/\bar{y}$) × 100.

Initial Investigation of Sample Preparation

A solvent study was first performed by comparing assay values of 0.5 g goldenseal root powder extracted ultrasonically for 30 min with 20.0 mL of methanol:water solvents, in ratios ranging from 50:50 to 100:0 at ambient temperature and 50°C. Then, the two-part procedure of Anderson and Burney(8) for validating the efficiency of simple extractions of analytes from herb samples was utilized. It consisted basically of a sample size study followed by a repeated extraction study.

In the first part, the maximum sample weight that could be extracted with a given extraction volume without affecting recovery was determined. In the second part, single extraction results using the optimum weight were compared with multiple extraction results to estimate % extracted (or recovered) in the single extraction.

All the results were then compared with triplicate, between-day Soxhlet extraction results, obtained following the procedure delineated in the USP.(2) From this data, the multiple extraction procedure described below was selected.

Final Sample Preparation-Multiple Extractions

An accurately weighed 0.5-g sample of powder, extract, or mortar-and-pestle ground caplet/tablet, was placed in a 50-mL centrifuge tube. About 20 mL of methanol:water extraction solvent (70:30 to 90:10) was added to the tube and the tube placed in a sonicator for about 30 minutes. The tube was centrifuged and as much of the supernatant as possible was quantitatively transferred with extraction solvent washings to a 100-mL volumetric flask. The residue was broken up with a spatula and an additional 20 mL of extraction solvent was added, washing off the spatula. The tube was then sonicated again for 30 minutes. This procedure

was repeated until the goldenseal in the tube had been extracted 4 times, with all of the extractions collected in the 100-mL volumetric flask, which was then diluted to volume with extraction solvent.

Final Sample Preparation-Pressurized Liquid Extraction

A cleaned frit from a used Bakerbond solid-phase extraction cartridge was sandwiched between two filters cut from prefilters that came with Millipore HA filters. These disposable filters were placed on top of the stainless-steel filter located at the exit end of the stainless-steel extraction cell. A plug of cotton, followed by an accurately weighed 0.5-g sample and another plug of cotton, was placed in the cell. The remaining dead volume of the 9.7-mL cell was filled with glass beads. Smaller cells were tried, but were found to be inconvenient for loading the sample and removing the compressed plug after extraction. The cell was tightly closed and inserted into the oven for the final connection of the system. The inlet and outlet valves were opened, the pump set at 1500 psi constant pressure, and turned on. The flow outlet was directed to a 50-mL volumetric flask. As soon as steady flow commenced into the flask, the outlet valve was closed and the oven turned on.

Five minutes after the oven temperature reached 100°C, the outlet valve was opened until 10 mL of methanol:water (70:30 to 90:10) extraction solvent was expelled into the flask. The outlet valve was then closed for another 5-minute cycle. This was repeated for a total of 4 cycles. After the final cycle, the remaining solvent in the cell was purged into the flask with nitrogen flow. After cooling, the contents of the flask were diluted to volume with extraction solvent. The inlet and outlet of the cell were switched for each successive sample to avoid buildup of exhausted microparticles in the stainless-steel exit frit.

Instead of successive static cycles, an alternate procedure employing a single dynamic cycle was also used. It consisted of opening the outlet valve at 100°C and 1500 psi to obtain a flow of 1 to 3 mL/min until 40 mL was expelled. The pressure was relieved and remaining solvent flushed from the cell with nitrogen. The flask was then diluted to volume as before. Since the shut-off valve had to be frequently adjusted to maintain the flow rate, this high-pressure, high-temperature percolation technique was not extensively examined.

RESULTS AND DISCUSSION

Final method-development results are summarized in Table 2 and described below.

Table 2. Summary of Method-Development Results, Product 9 Root Powder

Procedure	n	mg/g Hydrastine	mg/g Berberine
Soxhlet Extraction	3	23.1 ± 0.6 (2.4%)	42.1 ± 0.8 (2.0%)
Single Ultrasonic Extraction	6	20.9 ± 0.1 (0.6%)	37.3 ± 0.5 (1.2%)
Multiple Ultrasonic Extractions	6	22.9 ± 0.3 (1.4%)	42.0 ± 0.6 (1.4%)
Pressurized Solvent Extraction 4 cycles	5	22.9 ± 0.5 (2.3%)	42.3 ± 0.9 (2.1%)
Pressurized Solvent Extraction from Figure 4	6	22.6 ± 0.6 (2.6%)	42.8 ± 0.5 (1.2%)

Preliminary Extraction Study

The results of the solvent study, presented in Figure 2, indicate methanol: water (70:30 to 90:10) to be the solvents of choice, with a slight advantage for berberine at 50°C. Note, however, that assay values for berberine were significantly below the values obtained with Soxhlet extraction listed in Table 2. The sample size study with methanol:water (70:30), shown in Figure 3, indicated 0.5 g to be a reasonable sample size. Again, the berberine assay values were below

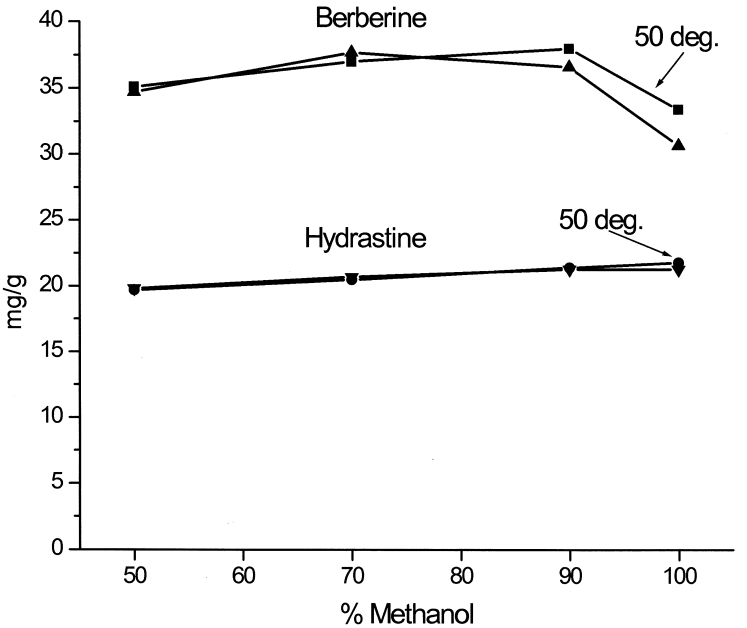

Figure 2. Assay values (mg/g) of goldenseal alkaloids *versus* methanol:water content for single ultrasonically-assisted extractions at ambient temperature and 50°C.

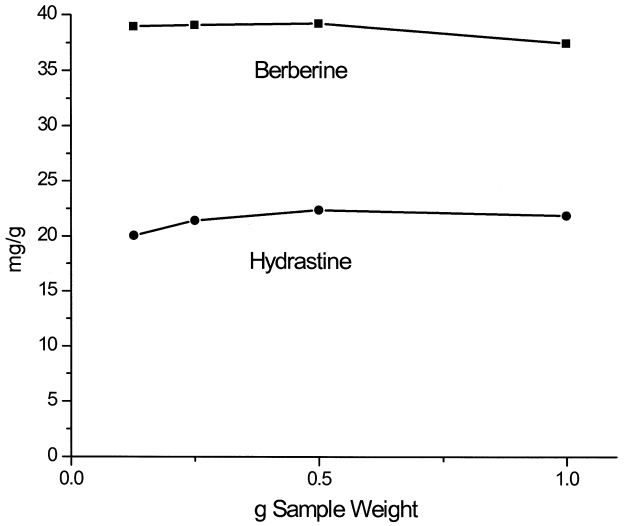

Figure 3. Assay values (mg/g) of goldenseal alkaloids *versus* sample weight for single ultrasonically-assisted extractions.

that obtained with Soxhlet extraction. Finally, 6 assays obtained by the single ultrasonic-assisted extraction procedure yielded results significantly less than that obtained by Soxhlet extraction, as shown in Table 2.

Multiple Extractions

Six assays obtained by taking 4 successive extractions as described in the Experimental Section, yielded the results in Table 2. Re-extraction of the residue after the fourth extraction yielded 0 for hydrastine and 0.3 mg/g or 0.7% of the initial assay for berberine. Thus, only about 87% of the berberine was extracted with a single extraction, but multiple extractions yielded results comparable to that for exhaustive Soxhlet extraction.

Pressurized Liquid Extraction

Assay values versus number of cycles were obtained for 3 extraction solvents—methanol:water (70:30, 90:10, and 100:0). The data, shown in Figure 4, indicates that the addition of water to the solvent enables exhaustive extraction of

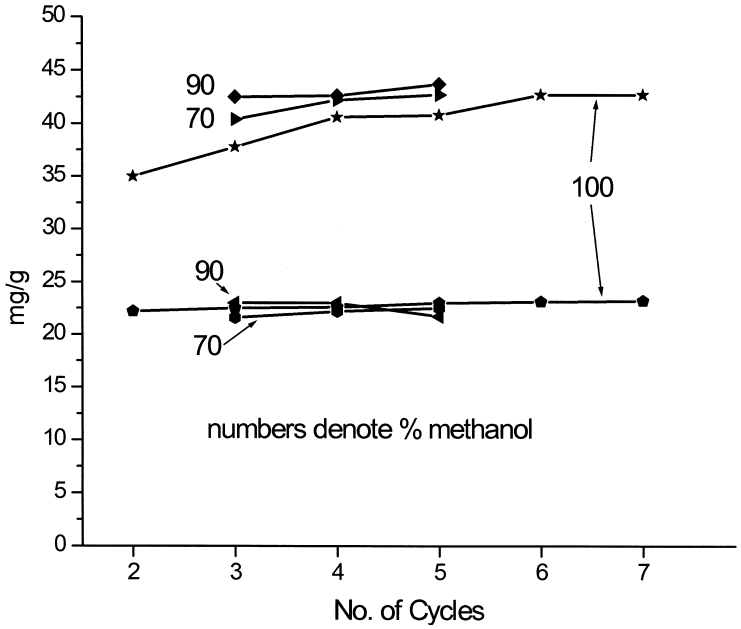

Figure 4. Assay values (mg/g) of goldenseal alkaloids *versus* number of cycles for pressurized liquid extractions.

berberine within 4 cycles. Hydrastine appears to be exhaustively extracted within 2 cycles. A 5-cycle reextraction of the residues yielded 0.0 to 0.1 mg/g (0 to 0.5%) remaining for hydrastine and 0.3 to 1.0 mg/g (0.7 to 2.3%) remaining for berberine in the last two cycles. Equivalent results were also obtained by 50-mL pressurized percolation experiments with methanol:water (70:30 or 90:10), but this technique was not extensively examined.

Five within-day assays of the goldenseal root powder obtained with 4 cycles of methanol:water (90:10) and 6 between-day assays taken from the last 2 cycles for the pressurized-liquid extraction data shown in Figure 4, yielded data consistent with previous data and Soxhlet extraction results, as shown in Table 2.

Replacing the final nitrogen purge with an equivalent flush with solvent from the syringe pump, also yielded results comparable to that for Soxhlet extractions and pressurized extractions utilizing the nitrogen purge. Thus, the nitrogen tank did not appear to be necessary and was removed from the system for later experiments. Increasing the pressure from 1500 psi to 2500 psi and/or increasing the temperature from 100°C to 125°C, aided the extraction only minimally.

Assay of Goldenseal Products

Assay results (average of 2 or more) of one tablet and 7 capsule formulations using the pressurized liquid extraction technique, are shown in Table 3. Assay values seem to be reasonably consistent among the capsule formulations. Products 2 and 4 are "standardized" by weight only, while product 5 claims 5% total alkaloids. The sum of hydrastine and berberine assay values yields 6.6% for this sample. Product 8, a mixture of extract and root powder in which both were assayed by capillary electrophoresis at a contract laboratory to determine a final value for the finished product, claims 8.3 mg hydrastine and 12.5 mg berberine per unit (± 10% for each). The measured results of 8.5 and 13.2 mg/unit, respectively, are in reasonable agreement considering the difference in techniques, reference standard purity, possible differences in extraction efficiency (especially for berberine), etc. The one-tablet formulation, product 3, has relatively low concentrations of alkaloids, mainly because a 900 mg tablet has, according to the label, only 80 mg of goldenseal. Hydrastine seems to be missing from this product, as shown by the chromatogram of this product superimposed on a chromatogram of an appropriately diluted goldenseal root powder (Figure 5). Either peak eluting near the retention time of hydrastine, has a spectrum similar to the other but significantly different from that of hydrastine, as shown in Figure 6. This indicates that the sample might not contain goldenseal but rather some other berberine-containing herb.

Table 3. Assay of Commercial Goldenseal Products for Hydrastine and Berberine Alkaloids

	Hydrastine		Berberine	
Product no.	mg/g	mg/unit[a]	mg/g	mg/unit[a]
1	26.4	15.3	34.4	20.0
2	21.5	11.7	28.3	15.4
3[b]	0	0	3.9	3.5
4[b]	27.0	14.2	35.9	18.9
5[b]	25.8	14.9	40.3	23.2
6	23.3	12.0	32.3	16.6
7	19.3	10.3	28.4	15.2
8	16.5	8.5	25.7	13.2

[a]Calculated from average unit weights.

[b]Label lists excipients in addition to gelatin support, extract and/or plant powder.

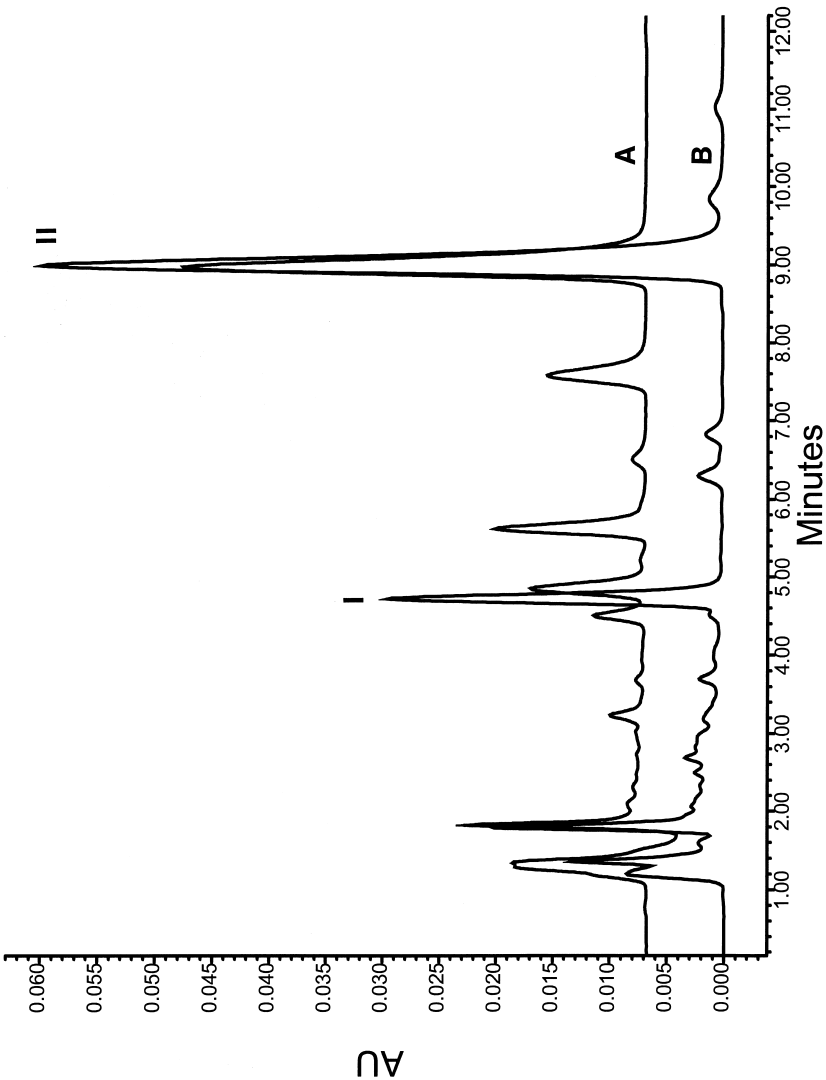

Figure 5. Chromatogram of Product 3 (A) superimposed on a chromatogram of goldenseal root powder (B), showing hydrastine (I) and berberine (II).

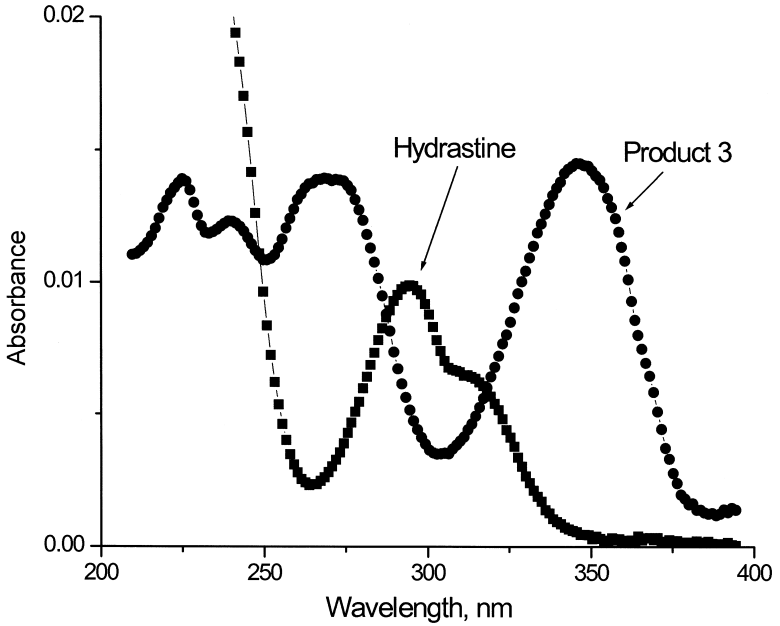

Figure 6. On-the-fly spectra of hydrastine peak and Product 3 peak found at retention time nearest to that for hydrastine.

SUMMARY

Both pressurized liquid extraction and multiple extractions provide significant time-savings over Soxhlet extraction and yield comparable results. Multiple extractions reduce the time for preparation of a single sample from 6 hours or more for Soxhlet extraction, to slightly more than 2 hours and avoid manipulation of cumbersome glassware and heating and cooling equipment. The multiple extraction technique is also more amenable to multiple samples. Pressurized liquid extraction further reduces extraction time to 30 minutes for a single sample and requires much less manual manipulation than the multiple extraction technique. For routine assay of many samples, commercial multicell automated pressurized liquid extractors might be worth the initial high cost.

REFERENCES

1. Murray, M.T.; Pizzorno, Jr., J.T. *Hydrastis canadensis* (Goldenseal) and Other Berberine-Containing Botanicals in *Textbook of Natural Medicine*,

2nd Ed.; Pizzorno, Jr., J.T., Murray, M.T. Eds.; Churchill: Livingstone, 1999; 775-781.
2. Goldenseal. Pharmacopeial Forum **2000**, *26* (4), 944-948.
3. Leone, M.G.; Cometa, M.F.; Palmery, M.; Saso, L. HPLC Determination of the Major Alkaloids Extracted from *Hydrastis canadensis L*. Phytother. Res. **1996**, *10* (Suppl.1), s45-s46.
4. Unger, M.; Stöckigt, J. Improved Detection of Alkaloids in Crude Extracts Applying Capillary Electrophoresis with Field Amplified Sample Injection. J. Chromatogr. **1997***, 791*, 323-331.
5. Richter, B.E. The Extraction of Analytes from Solid Samples Using Accelerated Solvent Extraction. LC-GC **1999**, *17* (6s), s22-s27.
6. Benthin, B.; Danz, H.; Hamburger, H. Pressurized Liquid Extraction of Medicinal Plants. J. Chromatogr. **1999**, *837*, 211-219.
7. Ong, E.-S.; Woo, S.-O.; Yong, Y.-L. Pressurized Liquid Extraction of Berberine and Aristolochic Acids in Medicinal Plants. J. Chromatogr. **2000**, *904*, 57-64.
8. Anderson, M.L.; Burney, D.P. Validation of Sample Preparation Procedures for Botanical Analysis. J. of AOAC Int. **1998**, *81* (5), 1005-1010.
9. Cardone, M.J.; Palermo, P.J.; Sybrandt, L.B. Potential Error in Single-Point-Ratio Calculations Based on Linear Calibration Curves with a Significant Intercept. Anal. Chem. **1980**, *52*, 1187-1191.

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