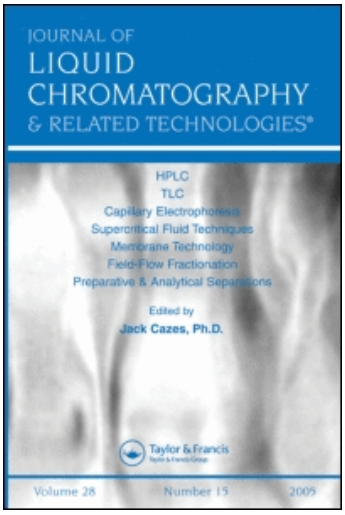
This article was downloaded by:
On: *24 January 2011*
Access details: *Access Details: Free Access*
Publisher *Taylor & Francis*
Informa Ltd Registered in England and Wales Registered Number: 1072954 Registered office: Mortimer House, 37-41 Mortimer Street, London W1T 3JH, UK



Journal of Liquid Chromatography & Related Technologies
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

HPLC ASSAY OF *ECHINACEA PURPUREA*/GOLDENSEAL (*HYDRASTIS CANADENSIS*) COMBINATION FORMULATIONS FOR PHENOLIC ACIDS, ALKYLAMIDES, AND ALKALOIDS

Gary W. Schieffer[a]; Michael Kohn[a]
[a] Graham Development, Inc., Oneonta, NY, U.S.A.

Online publication date: 05 July 2002

To cite this Article Schieffer, Gary W. and Kohn, Michael(2002) 'HPLC ASSAY OF *ECHINACEA PURPUREA*/GOLDENSEAL (*HYDRASTIS CANADENSIS*) COMBINATION FORMULATIONS FOR PHENOLIC ACIDS, ALKYLAMIDES, AND ALKALOIDS', Journal of Liquid Chromatography & Related Technologies, 25: 2, 263 — 274
To link to this Article: DOI: 10.1081/JLC-100108744
URL: http://dx.doi.org/10.1081/JLC-100108744

PLEASE SCROLL DOWN FOR ARTICLE

Full terms and conditions of use: http://www.informaworld.com/terms-and-conditions-of-access.pdf

This article may be used for research, teaching and private study purposes. Any substantial or systematic reproduction, re-distribution, re-selling, loan or sub-licensing, systematic supply or distribution in any form to anyone is expressly forbidden.

The publisher does not give any warranty express or implied or make any representation that the contents will be complete or accurate or up to date. The accuracy of any instructions, formulae and drug doses should be independently verified with primary sources. The publisher shall not be liable for any loss, actions, claims, proceedings, demand or costs or damages whatsoever or howsoever caused arising directly or indirectly in connection with or arising out of the use of this material.

J. LIQ. CHROM. & REL. TECHNOL., 25(2), 263–274 (2002)

HPLC ASSAY OF *ECHINACEA PURPUREA*/GOLDENSEAL (*HYDRASTIS CANADENSIS*) COMBINATION FORMULATIONS FOR PHENOLIC ACIDS, ALKYLAMIDES, AND ALKALOIDS

Gary W. Schieffer* and Michael Kohn

Graham Development, Inc., 179 River Street, Oneonta, NY 13820, USA

Downloaded At: 09:11 24 January 2011

ABSTRACT

A procedure involving anion-exchange solid-phase extraction is used to separate hydrastine and berberine alkaloids found in goldenseal (*Hydrastis canadensis*) from caffeic (phenolic) acids found in both *Echinacea purpurea* and goldenseal, prior to HPLC assay of *Echinacea purpurea*/goldenseal combination formulations. *Echinacea* alkylamides are separated from the phenolic acids and determined. Several commercial products are assayed.

*Corresponding author.

Copyright © 2002 by Marcel Dekker, Inc. www.dekker.com

ORDER REPRINTS

INTRODUCTION

Echinacea is a well-known herb that is employed primarily for stimulating the immune system. Goldenseal (*Hydrastis canadensis*) also possesses immunostimulating as well as antibiotic, anticonvulsant, and other pharmacological activity (1). Formulations frequently appear in the marketplace containing mixtures of the two herbs ranging from less than 20% to nearly 60% goldenseal. To create a high quality, consistent combination product, it is desirable to provide a suitable assay for presumed therapeutically active ingredients in each herb raw material prior to formulation. In addition, to ensure consistency and guarantee target label claims, it is also desirable to assay the final product.

As found on the shelf, *Echinacea* formulations that are "standardized" (other than those merely standardized by mass of total herb) are generally assayed to guarantee at least a portion of the preparation contains 3 or 4% phenolic compounds. These are frequently total phenolic acids, as measured by non-selective spectrophotometric procedures. Otherwise, caftaric acid (2-*O*-caffeoyltartaric acid), cichoric acid (2,3-*O*-dicaffeoyltartaric acid), and other caffeic- (caffeoyl- or phenolic-) acid derivatives for *E. purpurea* and caftaric acid, echinacoside, and other caffeic-acid derivatives for *E. angustifolia* are measured by HPLC (2–4). Some researchers (2) feel that lipophilic alkylamides, of which the isomeric pair dodeca-2E, 4E, 8Z, 10 E/Z tetraenoic acid isobutylamides is the most ubiquitous, are as important therapeutically as the more polar caffeic-acid derivatives. A method was recently presented in which caffeic-acid derivatives were quantitatively separated from alkylamides by C_{18} solid-phase extraction (SPE), so that each could be determined using separate HPLC procedures (3). Caftaric acid, cichoric acid, and other phenolic acids in *Echinacea purpurea* were allowed to pass through a C_{18} SPE column unretained, while alkylamides were retained and eluted later.

Goldenseal is normally standardized with respect to the isoquinoline alkaloids hydrastine and berberine. The USP delineates lower limits of 2.0% for the former and 2.5% for the latter in root powder and provides an HPLC method preceded by Soxhlet extraction (5). Another method involving Soxhlet extraction, provided an HPLC separation of hydrastine, berberine, and three other minor goldenseal alkaloids for eventual transfer to LC/MS analysis (6). Recently, a method for hydrastine and berberine in goldenseal involving either pressurized-liquid extraction or multiple ultrasonically-assisted extractions prior to the USP HPLC procedure, was presented (7). Solutions were injected directly without solid-phase extraction.

Unfortunately, assay procedures become more problematic when appreciable quantities of goldenseal are combined with *Echinacea.* In our experience, assay results for hydrastine and berberine do not appear to change when *Echinacea purpurea* is added to an equivalent mass of goldenseal, but a

Downloaded At: 09:11 24 January 2011

Copyright © Marcel Dekker, Inc. All rights reserved.

MARCEL DEKKER, INC.
270 Madison Avenue, New York, New York 10016

ORDER REPRINTS

noticeable loss in resolution occurs after just a few injections. Phenolic-acids assay for *Echinacea*, when goldenseal is present, does present a potential problem. Serious interference was not noticed for an apparently relatively low content of goldenseal in an *Echinacea* sample, (3) however, closer examination revealed that hydrastine and, especially berberine, will potentially interfere, as shown in Figure 1. Here, chromatograms obtained by separately assaying 0.5 g *Echinacea purpurea* and 0.5 g goldenseal samples using the previously described C_{18} SPE procedure (3) are compared. As shown, berberine, which is not completely retained by the C_{18} SPE column (30% loss), yielded a peak that would potentially coelute with cichoric acid. In addition, phenolic acids, especially chlorogenic acid, are prevalent in goldenseal. Thus, if prior to formulation, just phenolic acids are assayed in the *Echinacea* raw material and alkaloids measured in the goldenseal raw material, the assay of phenolic or caffeic acids for the final product will be higher than expected. Goldenseal raw material also needs to be assayed for phenolic acids.

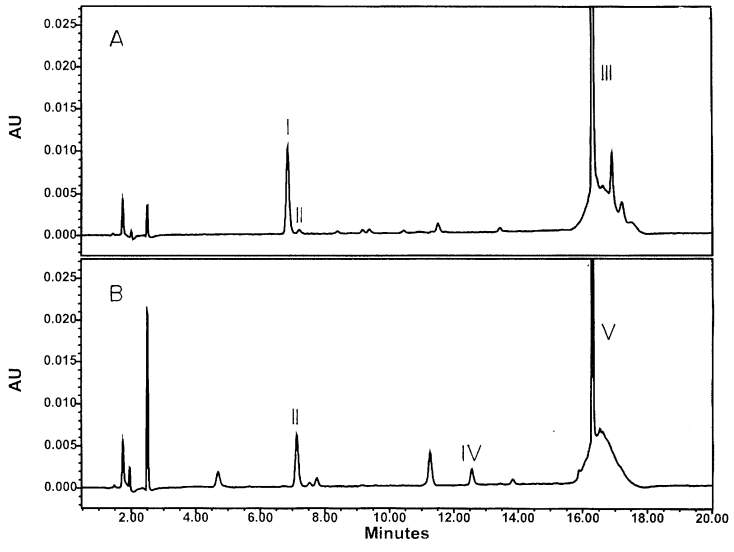


Figure 1. Expanded-scale chromatograms of 0.5 g *Echinacea purpurea* root powder (A) and 0.5 g goldenseal root powder (B), using the C_{18} SPE procedure outlined previously (3) for phenolic acids in *Echinacea*: I, caftaric acid, II, chlorogenic acid, III, cichoric acid, IV, hydrastine, V, berberine. Hump starting at about 16 minutes is a gradient artifact. 330 nm detection wavelength.

Downloaded At: 09:11 24 January 2011

Copyright © Marcel Dekker, Inc. All rights reserved.

MARCEL DEKKER, INC.
270 Madison Avenue, New York, New York 10016

ORDER REPRINTS

Anion-exchange solid-phase extraction was successfully used to separate and quantitate some common phenolic acids in *Echinacea* (8). In addition, preliminary work indicated that caftaric and cichoric acids could similarly be separated and assayed (3). The acids were loaded onto the anion-exchange column with either a methanol : sodium bicarbonate (15 : 85) solution (7) or methanol : water (70 : 30) solution (3) and quantitatively eluted with methanol : 0.2 M aqueous phosphoric acid (1 : 1) solution. The goal of this work was to use a similar anion-exchange SPE procedure to allow assay of *Echinacea purpurea*/goldenseal mixtures for caffeic (phenolic) acids, alkylamides, and alkaloids. Caffeic acids and other phenolic acids from both *Echinacea* and goldenseal would be quantitatively retained on the anion-exchange SPE column, while alkylamides from *Echinacea* and the alkaloids hydrastine and berberine from goldenseal would be allowed to completely pass through. Then, separate HPLC methods would follow.

Downloaded At: 09:11 24 January 2011

EXPERIMENTAL

Reagents and Chemicals

Chlorogenic acid, dodeca-2E, 4E, 8Z, 10E/Z tetraenoic acid isobutylamides, berberine chloride, and hydrastine standards described previously (3,7) were used. The following *Echinacea*/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) Eckerd Premium Echinacea and Goldenseal (9B04789), Eckerd Drug Co., Clearwater, FL; (2) Eckerd Echinacea and Goldenseal (8N02038); (3) Spring Valley Echinacea–Golden Seal Complex (N102021), NaturPharma, American Fork, UT; (4) Sundown Echinacea & Goldenseal Complex (944163), Sundown Vitamins, Boca Raton, FL; (5) Scientific Herbals Sinu-Free (GEG0101B), Graham Development, Inc., Oneonta, NY; (6) Goldenseal Root Powder (7940C), Stryka Botanics Co., Inc., Belle Mead, NJ; (7) *Echinacea purpurea* Root Powder (44035–50), Starwest Botanicals Inc., Rancho Cardova, CA; (8) *Echinacea angustifolia* Root Powder (45025–51), Starwest Botanics Co., Inc. Products 6, 7, and 8 root powders were used for method development. All products were assayed prior to the posted expiration date. All other reagents were HPLC or ACS quality, and were used without further purification.

Copyright © Marcel Dekker, Inc. All rights reserved.

MARCEL DEKKER, INC.
270 Madison Avenue, New York, New York 10016

ORDER REPRINTS

Apparatus

Either Varian Bond Elute, Bakerbond, or Strata C_{18} SPE columns and Bakerbond or Strata Quaternary Amine anion-exchange SPE columns, all with a 3-cc/500 mg bed mass were used with a Burdick & Jackson vacuum manifold. All of the columns were conditioned with 2 mL of methanol followed by 2 mL of water prior to use.

The HPLC systems and standard preparation used for Goldenseal and *Echinacea purpurea* are described in the separate publications (3,7). The HPLC system used for phenolic acids is similar to that presented by the Institute of Nutraceutical Advancement (INA) on the internet (4), while that for goldenseal alkaloids is based on the system presented in the USP (5).

Downloaded At: 09:11 24 January 2011

Sample Preparation

The main strategy was to assay *Echinacea purpurea* and goldenseal root-powder raw material samples separately, according to the previously validated procedures (3,7). Then, the raw material samples were to be combined in a 1 : 1 ratio and reassayed by the new procedure.

Since preliminary trials using pressurized liquid extraction (7) yielded erratic results for phenolic acids in *Echinacea* and alkaloids in goldenseal whenever *Echinacea* was present in the sample, the samples were extracted with multiple extractions as described previously for goldenseal (7). Either 0.5 g of *Echinacea purpurea* root powder, 0.5 g of goldenseal, or a mixture of 0.5 g of each were placed in a 50-mL centrifuge tube. About 20 mL of methanol : water (70 : 30) extraction solvent 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 sample 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.

For the samples containing just *Echinacea purpurea* root powder, 2.0 mL of each sample solution was loaded onto a C_{18} SPE column as described previously (3). Basically, phenolic acids were collected in the effluent after load and wash with 2 mL of methanol : water (30 : 70), while the retained alkylamides were eluted separately with acetonitrile : water (90 : 10). Sample solutions containing just goldenseal root powder were injected into the alkaloid HPLC

Copyright © Marcel Dekker, Inc. All rights reserved.

MARCEL DEKKER, INC.
270 Madison Avenue, New York, New York 10016

ORDER REPRINTS

system (7) directly. One goldenseal root powder sample solution was taken through the C_{18} SPE procedure for *Echinacea* with subsequent HPLC examination for phenolic acids (3).

Samples containing the *Echinacea purpurea*/goldenseal mixture were processed differently. After the initial multiple extraction, 2.0 mL of the final solution was loaded onto an anion-exchange SPE column and washed with 2 mL of methanol : water (30 : 70), collecting the load and wash in a 5-mL volumetric flask, which was then diluted to volume with the wash solvent. This solution was assayed for hydrastine and berberine (6) and alkylamides (3) by the separate HPLC procedures. The anion-exchange SPE column was then eluted with 5 mL of methanol : 0.2 M aqueous phosphoric acid (1 : 1), collecting the effluent in a 10-mL volumetric flask. This was then diluted to volume with 0.1 M aqueous sodium phosphate solution and assayed for phenolic acids (3). A goldenseal sample alone was also assayed for phenolic acids by this procedure.

Downloaded At: 09:11 24 January 2011

RESULTS AND DISCUSSION

Hydrastine and Berberine

Results for separate assays of goldenseal alone for the alkaloids hydrastine and berberine using direct injection (7) and for 1 : 1 goldenseal/*Echinacea purpurea* mixtures using anion-exchange SPE, are shown in Table 1 and Figure 2. The between-day assays are in reasonable agreement. The difference in signal for the two chromatograms in Figure 2 is due to the 2.0- to 5.0-mL SPE dilution for the goldenseal/*Echinacea purpurea* sample. Notice the relative decrease in signal for peaks near the solvent front. This decrease is most likely due to removal of phenolic acids by the anion-exchange SPE procedure.

Table 1. Assay (mg/g of Individual Herb) of Hydrastine and Berberine in Goldenseal With and Without *Echinacea purpurea* Present

Condition	n	mg/g Hydrastine	mg/g Berberine
Goldenseal alone, direct injection	6	18.2 ± 0.2 (1.0%)	24.7 ± 0.4 (1.4%)
Goldenseal/*Echinacea purpurea* (1:1), anion-exchange SPE	6	18.8 ± 0.5 (2.6%)	24.1 ± 0.8 (3.4%)

Copyright © Marcel Dekker, Inc. All rights reserved.

MARCEL DEKKER, INC.
270 Madison Avenue, New York, New York 10016

ORDER REPRINTS

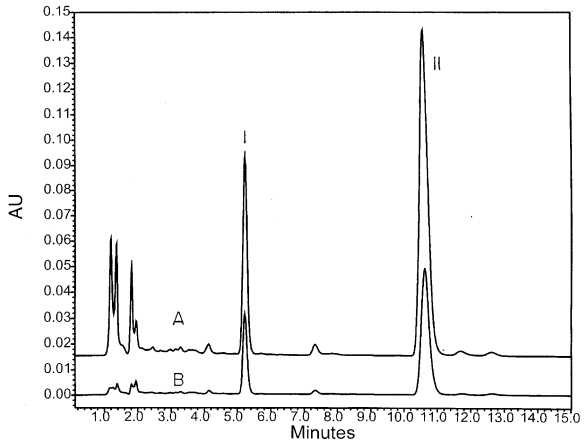


Figure 2. Chromatograms of 0.5 g goldenseal root powder (A) and 0.5 g goldenseal root powder/0.5 g *Echinacea purpurea* root powder (B) for alkaloids: I, hydrastine and II, berberine. 235 nm detection wavelength.

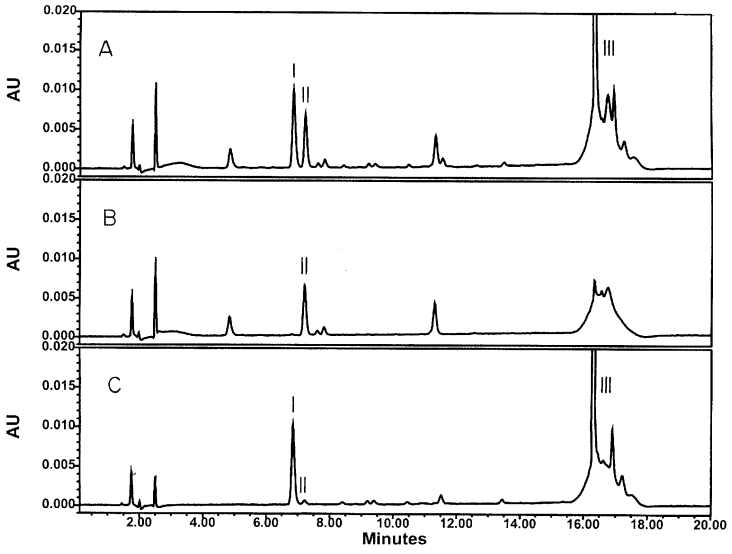


Figure 3. Expanded-scale chromatograms of 0.5 g goldenseal root powder/0.5 g *Echinacea purpurea* root powder (A), 0.5 g goldenseal root powder (B), and 0.5 g *E. purpurea* root powder (C) for phenolic acids. A and B involve anion-exchange SPE, C involves C_{18} SPE. I, caftaric acid, II, chlorogenic acid, III, cichoric acid. 330 nm detection wavelength.

Downloaded At: 09:11 24 January 2011

Copyright © Marcel Dekker, Inc. All rights reserved.

MARCEL DEKKER, INC.
270 Madison Avenue, New York, New York 10016

ORDER REPRINTS

Table 2. Assay (mg/g of Individual Herb) of Caffeic (Phenolic) Acids in Goldenseal, and Goldenseal/*Echinacea purpurea* Mixtures Using Anion-Exchange SPE and Assay of Caffeic Acids in *Echinacea purpurea* Using C_{18} SPE

Condition	n	mg/g Caftaric Acid	mg/g Chlorogenic Acid	mg/g Chicoric Acid	mg/g Total Phenolic Acids[a]
Goldenseal alone	1	0.07	2.2	0.1	6.7
Goldenseal/*Echinacea purpurea* (1:1)	5	3.42 ± 0.02 (0.7%)	2.63 ± 0.03 (1.2%)	14.9 ± 0.1 (0.9%)	26.8 ± 0.2 (0.7%)
Echinacea purpurea	6	3.47 ± 0.05 (1.3%)	0.18 ± 0.01 (5.7%)	15.2 ± 0.1 (0.6%)	20.7 ± 0.5 (2.3%)

[a]Determined from sum of caftaric, chlorogenic, cichoric acid content as themselves plus that for all other eluting acids as cichoric acid (3).

Downloaded At: 09:11 24 January 2011

Phenolic Acids

Chromatograms of goldenseal and goldenseal/*Echinacea purpurea*, taken through the anion-exchange SPE procedure, and *Echinacea purpurea*, taken through the C_{18} SPE procedure (0.5 g of each individual root powder), are shown in Figure 3. The anion-exchange procedure effectively removed the hydrastine and berberine interference present in goldenseal. The low level of "chicoric acid" observed for the goldenseal sample in Table 2 may be due to trace amounts of residual berberine. The caftaric and chicoric acid results obtained for anion-exchange SPE of goldenseal/*Echinacea purpurea* (1 : 1) mixtures, are in reasonable agreement with that obtained for the C_{18} SPE procedure of *Echinacea purpurea* alone, as shown in Table 2. Since significant amounts of phenolic acids exist in goldenseal, the total phenolic acids assay for the *Echinacea purpurea*/goldenseal mixture is greater than that for the *Echinacea purpurea* alone. This necessitates an assay of phenolic acids in goldenseal raw material prior to preparing a mixture with *Echinacea purpurea*, if a product with a predictable amount of phenolic acids is desired.

If it is desired to mix goldenseal with *Echinacea angustifolia*, an extra injection will be required for assaying echinacoside, since the ester passes through the anion-exchange SPE column virtually unretained. The solution containing the goldenseal alkaloids, *Echinacea angustifolia* alkylamides, and echinacoside, collected after passing through the anion-exchange SPE column, would need to be injected in the phenolic-acids HPLC system in addition to the solution containing the phenolic acids separately eluted from the anion-exchange SPE column. Echinacoside, which is resolved from goldenseal alkaloids, would

Copyright © Marcel Dekker, Inc. All rights reserved.

Marcel Dekker, Inc.
270 Madison Avenue, New York, New York 10016

ORDER REPRINTS

be determined in the first injection, while the remaining phenolic acids would be determined in the later injection. The first solution would then be injected into the alkaloid HPLC system (7) to determine hydrastine and berberine and the alkylamide system (3) to determine alkylamides.

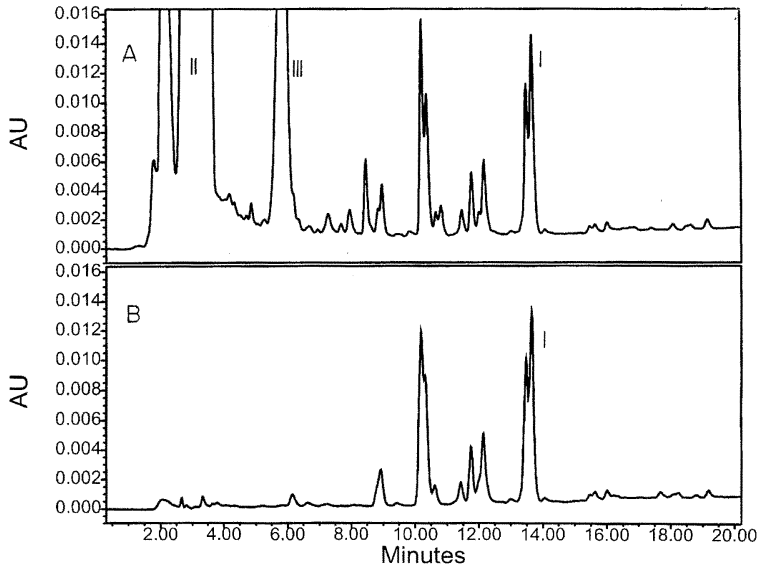


Figure 4. Chromatograms of 0.5 g *Echinacea purpurea* root powder/0.5 g goldenseal root powder (A) taken through the anion-exchange SPE procedure and 0.5 g *E. purpurea* (B) taken through the C_{18} SPE procedure for alkylamides: I, dodeca-2E, 4E, 8Z, 10 E/Z tetraenoic acid isobutylamides, II, berberine, III, hydrastine. 254 nm detection wavelength.

Table 3. Assay (mg/g of Individual Herb) of Alkylamides in *Echinacea purpurea* and Goldenseal/*Echinacea purpurea* Mixtures

Condition	n	mg/g Dodeca-2E, 4E, 8Z, 10 E/Z Tetraenoic Acid Isobutylamides	mg/g Total Alkylamides[a]
Echinacea purpurea alone	6	0.806 ± 0.006 (0.7%)	2.31 ± 0.04 (1.8%)
Goldenseal/*Echinacea purpurea* (1:1)	6	0.78 ± 0.01 (1.7%)	2.58 ± 0.08 (3.1%)

[a]Determined from sum of dodeca-2E, 4E, 8Z, 10 E/Z tetraenoic acid isobutylamides content and that for all other eluting peaks excluding hydrastine and berberine.

Downloaded At: 09:11 24 January 2011

Copyright © Marcel Dekker, Inc. All rights reserved.

ORDER REPRINTS

Alkylamides

Chromatograms of the 1 : 1 *Echinacea purpurea*/goldenseal mixture obtained using anion-exchange SPE and *Echinacea purpurea* alone using C_{18} SPE, are shown in Figure 4. Thus, goldenseal alkaloids will provide some interference with the total alkylamide assay, even if hydrastine and berberine are carefully screened out. Table 3 indicates reasonable agreement between the two procedures for dodeca-2E, 4E, 8Z, 10 E/Z tetraenoic acid isobutylamides, but the total alkylamide assay is about 12% higher for the mixture, if integration is started after elution of the hydrastine peak.

Table 4. Assay of Commercial *Echinacea*/Goldenseal Products for Hydrastine and Berberine Alkaloids

	Hydrastine			Berberine		
Product No.	mg/g	mg/unit[a]	Label Claim	mg/g	mg/unit[a]	Label Claim
1[b]	2.7	2.1	5 mg/unit total[c]	3.6	2.8	5 mg/unit total[c]
2[b]	0	0		19.4	9.2	
3	2.3	1.1		3.5	1.7	
4	3.2	1.0		4.1	1.3	
5	8.1	4.5	4.15 mg/unit[d]	12.7	7.0	6.25 mg/unit

[a]Calculated from average unit weights.
[b]Label lists excipients in addition to gelatin support, extract and/or plant powder.
[c]100 mg stantardized to 5% total alkaloids.
[d]Minimum value.

Table 5. Assay of Commercial *Echinacea*/Goldenseal Products for Phenolic Acids

	Chicoric Acid		Caftaric Acid	Chlorogenic Acid	Total Phenolic Acids		
Product No.	mg/g	mg/unit[a]	mg/g	mg/g	mg/g	mg/unit[a]	Label Claim
1	2.5	1.9	1.2	0.2	5.3[b]	4.1[b]	3 mg/unit[c]
2	2.9	1.4	1.6	0.1	7.8	3.7	
3	12.8	6.1	6.2	0.4	22.4	10.7	
4	6.7	2.2	4.1	0.4	13.7	4.5	
5	10.1	5.6	6.2	1.5	22.0	12.2	12 mg/unit

[a]Calculated from average unit weights.
[b]Not tested for echinacoside. Label lists both *E. purpurea* and *angustfolia.*
[c]75 mg standardized to 4% phenolic compounds.

Downloaded At: 09:11 24 January 2011

Copyright © Marcel Dekker, Inc. All rights reserved.

MARCEL DEKKER, INC.
270 Madison Avenue, New York, New York 10016

ORDER REPRINTS

Table 6. Assay of Commercial *Echinacea*/Goldenseal Products for Alkylamides

	Dodeca-2E, 4E, 8Z, 10 E/Z Tetraenoic Acid Isobutylamides		Total Alkylamides	
Product No.	mg/g	mg/unit[a]	mg/g	mg/unit[a]
1	0.06	0.04	0.12	0.09
2	0.16	0.08	0.24	0.11
3	0.37	0.18	0.54	0.26
4	0.11	0.04	0.18	0.06
5	2.81	1.56	4.24	2.34

[a]Calculated from average unit weights.

Downloaded At: 09:11 24 January 2011

Assay of *Echinacea*/Goldenseal Products

Assay results (average of 2 or more) of one caplet and 4 capsule formulations for hydrastine and berberine are shown in Table 4. Single-unit results for the sum of the two alkaloids range over a factor of 5. Assay results for the standardized products agree reasonably well with the label claims. Hydrastine seems to be missing from product 2. In fact, the chromatographic fingerprint and associated UV spectra are identical to that observed for a goldenseal sample from another manufacturer that also did not appear to contain hydrastine (7). This indicates that some goldenseal products might not contain goldenseal but rather some other berberine-containing herb.

Single-unit results for total phenolic acids differed by a factor of 3, as shown in Table 5. As before, assay results agreed reasonably well with label claims for the two standardized products. As shown in Table 6, alkylamide levels were relatively low for 4 of the products and high for one product. None of the products made label claims for alkylamides.

SUMMARY

Anion-exchange solid-phase extraction effectively separates hydrastine and berberine alkaloids from caffeic (phenolic) acids when *Echinacea purpurea* and goldenseal are mixed in a combination formulation. As a result, standardization parameters may be obtained and quality control assured for both herbs in the combination. Goldenseal contains a significant amount of phenolic acids, especially chlorogenic acid, which should be determined prior to

Copyright © Marcel Dekker, Inc. All rights reserved.

MARCEL DEKKER, INC.
270 Madison Avenue, New York, New York 10016

ORDER REPRINTS

formulation, in order to control the total phenolic acids in the final combination formulation. Alkylamides in the *Echinacea* may be determined, but total alkylamides may be slightly high due to interferences from minor alkaloids in the goldenseal. The main alkylamide isomeric pair dodeca-2E, 4E, 8Z, 10 E/Z tetraenoic acid isobutylamides may be determined without interference. *Echinacea angustifolia* may be used in place of *E. purpurea* but will require an additional step to determine echinacoside, an ester not retained by the anion-exchange column.

Some products purported to contain goldenseal may actually contain a berberine-containing herb other than goldenseal.

REFERENCES

1. Murray, M.T.; Pizzorno Jr., J.E. *Hydrastis Canadensis* (Goldenseal) and Other Berberine-Containing Botanicals. In *Textbook of Natural Medicine*, 2nd Ed.; Pizzorno Jr., J.E., Murray, M.T., Eds.; Churchill Livingstone: London, 1999, 775–781.
2. Bauer, R. *Echinacea*: *Biological Effects and Active Principles*. ACS Symp. Ser.; American Chemical Society: Washington, 1998. (Phytomed. Europe) **1998**, *691*, 140–157.
3. Schieffer, G.W. Validated HPLC Method for Caffeic-Acid Derivatives and Alkylamides in *Echinacea* Solid-Dosage Forms Using a Single Extraction Procedure. JANA **2000**, *3* (3), 67–81.
4. www.nutraceuticalinstitute.com/methods/echinacea.html (accessed July 2001).
5. Goldenseal. Pharmacopeial Forum **2000**, *26* (4), 944–948.
6. Weber, H.A.; Zart, M.K.; Ferguson, S.L.; Greaves, J.G.; Clark, A.P.; Harris, R.K.; Overstreet, D.; Smith, C. Separation and Quantitation of Isoquinoline Alkaloids Occurring in Goldenseal. J. Liq. Chrom. and Rel. Technol. **2001**, *24* (1), 87–95.
7. Schieffer, G.W.; Pfeiffer, K. Pressurized Liquid Extraction and Multiple Ultrasonically-Assisted Extractions of Hydrastine and Berberine from Goldenseal (*Hydrastis canadensis*) with Subsequent HPLC Assay. J. Liq. Chrom. and Rel. Technol., *in press*.
8. Glowniak, K; Zgórka, G; Kozyra, M. Solid-Phase Extraction and Reversed-Phase High-Performance Liquid Chromatography of Free Phenolic Acids in Some *Echinacea* Species. J. Chromatogr. **1996**, *730*, 25–29.

Received July 17, 2001
Accepted August 22, 2001
Manuscript 5620

Downloaded At: 09:11 24 January 2011

Copyright © Marcel Dekker, Inc. All rights reserved.

MARCEL DEKKER, INC.
270 Madison Avenue, New York, New York 10016

Downloaded At: 09:11 24 January 2011

Request Permission or Order Reprints Instantly!

Interested in copying and sharing this article? In most cases, U.S. Copyright Law requires that you get permission from the article's rightsholder before using copyrighted content.

All information and materials found in this article, including but not limited to text, trademarks, patents, logos, graphics and images (the "Materials"), are the copyrighted works and other forms of intellectual property of Marcel Dekker, Inc., or its licensors. All rights not expressly granted are reserved.

Get permission to lawfully reproduce and distribute the Materials or order reprints quickly and painlessly. Simply click on the "Request Permission/Reprints Here" link below and follow the instructions. Visit the U.S. Copyright Office for information on Fair Use limitations of U.S. copyright law. Please refer to The Association of American Publishers' (AAP) website for guidelines on Fair Use in the Classroom.

The Materials are for your personal use only and cannot be reformatted, reposted, resold or distributed by electronic means or otherwise without permission from Marcel Dekker, Inc. Marcel Dekker, Inc. grants you the limited right to display the Materials only on your personal computer or personal wireless device, and to copy and download single copies of such Materials provided that any copyright, trademark or other notice appearing on such Materials is also retained by, displayed, copied or downloaded as part of the Materials and is not removed or obscured, and provided you do not edit, modify, alter or enhance the Materials. Please refer to our Website User Agreement for more details.

Order now!

Reprints of this article can also be ordered at

http://www.dekker.com/servlet/product/DOI/101081JLC100108744