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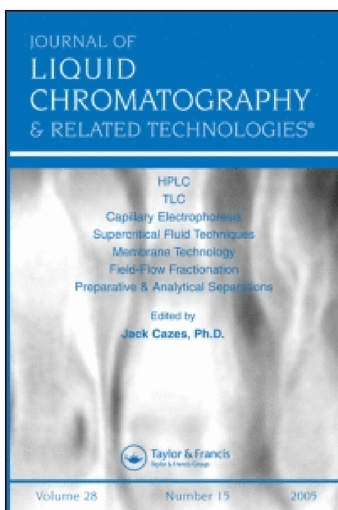

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SEPARATION AND QUANTITATION OF ISOQUINOLINE ALKALOIDS OCCURRING IN GOLDENSEAL

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ABSTRACT

An isocratic high-performance liquid chromatography (HPLC) method was developed for the analysis of hydrastinine, palmatine, berberine, hydrastine, and canadine, all alkaloid components known to be present in goldenseal root powder. Optimized separation was achieved on a Zorbax Eclipse-XDB column at 30°C using a mobile phase of 10 m*M* ammonium acetate/acetonitrile (70:30, v/v) at a flow rate of 1.0 mL/min with ultraviolet detection at 235 nm. The method showed linearity for palmatine, berberine, hydrastine, and canadine at approximately 4–400 μg/mL, while hydrastinine was linear at approximately 4–80 μg/mL. Method precision and robustness were investigated. An example of an HPLC analysis of goldenseal root powder extract is presented here also. These studies indicate that the method described here is usable for analysis of goldenseal extracts.

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INTRODUCTION

Goldenseal root powder is derived from the dried rhizome and root fibers of *Hydrastis canadensis* (family Ranulanceae). This indigenous North American perennial herb is widely cultivated, and its extracts have been used for a variety of medicinal purposes. Goldenseal has been reported to be one of the five best-selling herbal supplements among dietary supplements sold in the United States (1). *Hydrastis canadensis* has been reported to contain several isoquinoline alkaloids, including 2–4% hydrastine (2) and 2–3% berberine (2) by weight, as well as lesser amounts of canadine (2) and berberastine (3).

Several high-performance liquid chromatography (HPLC) methods have been published for the separation of berberine and hydrastine from goldenseal extracts (4,5), as well as for separating other structurally related components from natural sources (6–10). Methods that might be appropriate for liquid chromatography/mass spectrometry (LC/MS) analysis of concomitant separation of berberine, hydrastine, and canadine from goldenseal extracts have not been published.

This report presents an isocratic method for the separation of berberine, hydrastine, and canadine, along with two other closely related compounds, hydrastinine and palmatine. The separation was achieved on a Zorbax Eclipse-XDB column at 30°C using a mobile phase of 10 m*M* ammonium/acetonitrile (70:30, v/v) at a flow rate of 1.0 mL/min with ultraviolet (UV) detection at 235 nm.

The method showed linearity for palmatine, berberine, hydrastine, and canadine at approximately 4–400 μg/mL, while hydrastinine was linear at approximately 4–80 μg/mL. Method specificity, precision, and robustness were evaluated. Extracts of goldenseal root powder were also analyzed.

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EXPERIMENTAL

Chemicals and Reagents

Reference standards for berberine chloride dihydrate (berberine), hydrastinine hydrochloride (hydrastinine), and (1*R*, 9*S*,)-β-hydrastine hydrochloride (hydrastine) were obtained from Sigma Chemical Company (St. Louis, MO, USA). Palmatine chloride hydrate (palmatine) was obtained from Aldrich Chemical Company, Inc. (Milwaukee, WI, USA). Canadine (DL-tetrahydroberberine) was obtained from Apin Chemicals Limited (UK) (Abingdon, Oxon, UK).

HPLC grade acetonitrile and HPLC grade chloroform were obtained from Fisher Scientific (Pittsburgh, PA, USA) and Burdick and Jackson (Muskegon, MI, USA). U.S. Pharmacopoeia (USP) grade 190-proof ethanol was obtained from McCormick Distilling Company, Inc. (Weston, MO, USA). HPLC grade water

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(18 MΩ-cm) was obtained from an in-house Milli-Q system (Waters, Milford, MA, USA).

HPLC grade ammonium acetate was obtained from Fisher Scientific. USP grade acetic acid was obtained from Mallinckrodt, Inc. (St. Louis, MO, USA). The pH buffer calibration solutions were obtained from Ricca Chemical Company (Arlington, TX, USA) and EM Sciences (Gibbstown, NJ, USA). Goldenseal root powder was obtained from Quality Botanical Ingredients, Inc. (South Plainfield, NJ, USA).

Instrumentation and Equipment

Four HPLC systems were assembled for this investigation. System 1 was used to assess linearity of response using the columns selected. Systems 1, 2, 3, and 4 were used to assess robustness of the method. Chromatographic parameters and system suitability were determined with System 4.

HPLC system 1 consisted of two Waters 510 HPLC pumps, a Waters 680 controller, a Waters 717 Plus autosampler, a Spectra System 1000 UV detector (Thermo Separation Products, San Jose, CA, USA), a CH-30 column heater with a TC-50 controller (Eppendorf, Hamburg, Germany), and a Turbochrom data system (Perkin-Elmer, Norwalk, CT, USA).

HPLC system 2 consisted of two Waters 510 HPLC pumps, a Waters 680 controller, a Waters 717 Plus autosampler, a Spectra 100 UV detector (Spectra Physics, San Jose, CA, USA), an Eppendorf CH-30 column heater with an Eppendorf TC-50 controller, and a Perkin-Elmer Turbochrom data system.

HPLC system 3 consisted of a Waters Alliance 2690 separations module, a Waters 2487 UV detector, and a Perkin-Elmer Turbochrom data system.

HPLC system 4 consisted of a 1050 gradient pump and vacuum degasser (Hewlett-Packard, Wilmington, DE, USA), a Waters 717 Plus autosampler, a Thermo Separation Products Spectra System 1000 UV detector, an Eppendorf CH-30 column heater with an Eppendorf TC-50 controller, and a Perkin-Elmer Turbochrom data system.

Chromatographic separation was achieved at 30–35°C with a Zorbax Eclipse-XDB column, 5 μm, 250 mm × 4.6 mm inside diameter (Hewlett-Packard). Mobile phase A for HPLC systems 1 and 2 consisted of 10 m*M* ammonium acetate (adjusted to pH ∼4.8)/acetonitrile (90:10, v/v), while mobile phase B consisted of 10 m*M* ammonium acetate (adjusted to pH ∼4.8)/acetonitrile (10:90, v/v).

The eluting mobile phase was A/B (74:26, v/v), which corresponds approximately to 69% of 10 m*M* ammonium acetate (adjusted to pH ∼4.8)/31% acetonitrile. Mobile phase A for HPLC systems 3 and 4 consisted of 10 m*M* ammonium acetate (adjusted to pH ∼4.8)/acetonitrile, while mobile phase B consisted of acetonitrile. The eluting mobile phase was A/B (70:30, v/v). A flow rate

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of 1.0 mL/min was used for all systems with a 10-μL injection volume and UV detection at 235 nm. The total run time was 45 mins.

Goldenseal root powder was extracted with a combination heating mantle (RX series; Glas-Col, Terre Haute, IN, USA), fitted with 200-mL round-bottom flasks, Soxhlet extraction tubes, and condensers (PYREX, Corning, Inc., Corning, NY, USA). PYREX extraction thimbles (170–200 μm, extra coarse porosity, 30-mL) were used in the Soxhlet apparatus. Whatman no. 42 ashless filter paper, 11.0 cm (Whatman International, Inc., Maidstone, Kent, UK), was used to filter the extracts.

A Büchi model RE 120 rotary evaporator (Brinkmann Instruments, Inc., Westbury, NY, USA) was used to concentrate the extracts. Residues were concentrated with an N-EVAP 112 nitrogen evaporator (Organomation Associates, Inc., Berlin, MA, USA) with ultra high-purity nitrogen (Helget Gas Products, Kansas City, MO, USA).

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Standard Preparation

Stock solutions of hydrastinine, palmatine, berberine, hydrastine, and canadine were prepared by weighing ~5 mg of each reference standard and quantitatively transferring the sample into individual 10-mL amber volumetric flasks. To obtain dissolution of the berberine, approximately 1 mL of water was added to the flask, which was then diluted to volume with acetonitrile, and thoroughly mixed. All other stock standards were dissolved, diluted to volume with acetonitrile, and thoroughly mixed.

For determination of chromatographic parameters, a mixed standard was prepared by transferring 200 μL of each of the stock solutions into a 1-dram amber vial, adding 1000 μL of water, and mixing well. Components of the mixed standard were at the following concentrations: 41.7 μg/mL hydrastinine, 47.1 μg/mL palmatine, 36.4 μg/mL berberine, 47.0 μg/mL hydrastine, and 49.6 μg/mL canadine.

For linearity studies, aliquots of each stock solution were diluted with water to give solutions containing approximately 400, 300, 200, 100, and 50 μg/mL of each reference standard. The 50-μg/mL solutions were diluted further with water/acetonitrile (50:50, v/v) to give individual standards containing approximately 40, 30, 20, 10, or 5 μg/mL of each component. For each goldenseal component, linearity calculations were based on peak areas at the concentrations prepared.

Goldenseal Root Powder Extraction

An individual aliquot of root powder (5 g) was Soxhlet-extracted with approximately 200 mL of hexane/chloroform/ethanol (1:1:4, v/v) for approximately 24 h.

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The extract was collected and filtered using filter paper in a Büchner funnel. Solvent was removed by rotary evaporation, and the resulting residue was transferred to a preweighed vial. This residue was dried with nitrogen stream, and the vial was re-weighed to determine the amount of extractable residue. Portions of the residue were prepared for HPLC analysis, as outlined below, and the remaining residue was stored under frozen conditions.

Preparation of Residues for HPLC Analysis

Goldenseal residue (preparation described above) was dissolved in 1–4 mL of a chloroform/ethanol mixture. Using a micropipette, a calculated volume containing 2 mg of residue was transferred to a vial, the solvent was removed with a nitrogen stream, and the resulting residue was re-dissolved in 2 mL of water/acetonitrile (50:50, v/v).

RESULTS AND DISCUSSION

No reports were found in the scientific literature describing simultaneous separation of hydrastinine, palmatine, berberine, hydrastine, and canadine. Our initial studies to develop an isocratic method suitable for transfer to a LC/MS analysis involved the use of various octadecylsilane columns with mobile phases containing acetonitrile and trifluoroacetic acid or ammonium acetate buffers.

Initial screening of the chromatographic behavior of these components indicated that use of a Zorbax Eclipse-XDB column, with a mobile phase containing acetonitrile and 10 mM ammonium acetate buffer at pH ∼4.8, gave the most reproducible results. However, it must be noted that variations in retention times were observed with different buffer preparations. Figure 1 shows a typical chromatogram of a mixed standard of known goldenseal components.

It was discovered during these initial investigations, that while palmatine and berberine eluted more quickly with an increase in column temperature, hydrastine and canadine were retained longer on the column. In addition, some water (at least 10%) was required in the diluent to obtain good peak shape for palmatine and berberine.

Linearity and System Suitability

Using HPLC system 1, linear ranges for each goldenseal component with mobile phase A/mobile phase B (74:26, v/v) and a column temperature of 35°C are shown in Table 1. [Mobile phase A was 10 m*M* ammonium acetate/acetonitrile

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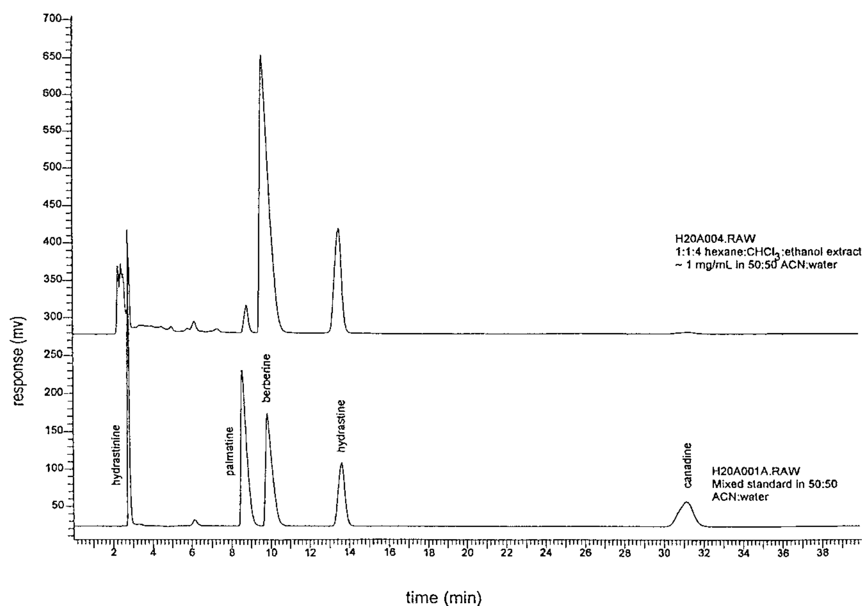

Figure 1. Chromatograms of mixed goldenseal alkaloid standards and goldenseal extract.

(90:10, v/v) and mobile phase B was10 m*M* ammonium acetate/acetonitrile (10:90, v/v).]

A plot of response versus concentration for each standard is shown in Figure 2. Chromatographic parameters and system suitability were determined

Table 1. Analytical Figures of Merit for Standards of Known Goldenseal Components

Component	Linear Range (μg/mL)	r	System Suitability[a]	k'^{b}	N^{b}	T^{b}	$R^{b,c}$
Hydrastinine	4.2–83	0.9983	0.38	0.20	5250	1.46	
Palmatine	4.7–378	0.9998	0.36	2.51	3234	1.87	1.96
Berberine	3.6–291	0.9998	0.37	3.02	3466	1.94	
Hydrastine	4.7–376	0.9996	0.30	2.99	15036	1.22	
Canadine	5.0–397	0.9988	0.16	9.96	18509	1.31	

[a]Results of system suitability studies are presented as % relative standard deviation of the peak areas of six replicate injections of the mixed standard.
[b]k' = capacity factor; N = theoretical plates; T = tailing factor; R = resolution; all were calculated according to USP guidelines.
[c]Resolution between palmatine and berberine peaks.

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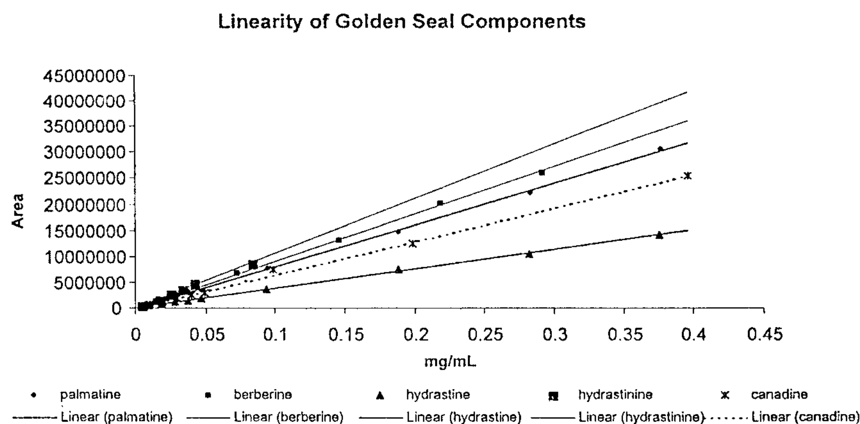

Figure 2. Linearity results plotted for standards of known goldenseal components.

using HPLC system 4 with 10 m*M* ammonium acetate/acetonitrile (70:30, v/v) and a column temperature of 30°C.

Robustness

The robustness of the method was evaluated using the four previously described HPLC systems and two Zorbax Eclipse-XDB columns. Using HPLC systems 1, 2, 3, and 4 and the mixed standard solution, optimum column temperature, aqueous/organic ratio, and the pH of the ammonium acetate solution were investigated. During column temperature investigations, it was determined that column temperatures ranging from ambient (∼22°C) to 40°C gave adequate chromatography. However, increasing the column temperature to 45°C resulted in inadequate resolution between palmatine and berberine, while fluctuations in ambient temperatures resulted in drifting of the retention times of both hydrastine and canadine.

Using a controlled column temperature of 30°C, adequate chromatographic results were observed with aqueous/organic ratios ranging from 76:24 (v/v) to 64:36 (v/v). However, raising the column temperature to 35°C led to a narrowing of the suitable aqueous/organic ratios to 74:26 (v/v)–66:34 (v/v). Using an aqueous/organic ratio of 70:30 (v/v) and a column temperature of 30°C, it was determined that the pH of the 10 m*M* ammonium acetate buffer could be adjusted between 4.7 and 5.1 and yield adequate separation and elution of all components within 45 min. Table 2 summarizes the parameters that yield acceptable chromatographic separation of the mixed standard.

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Table 2. Chromatographic Parameters of Goldenseal Standard Separation (Robustness Studies)

	Maximum	Minimum
Column temperature (°C)	40	Ambient
10 m*M* ammonium acetate/acetonitrile ratio (v/v); column temperature = 30°C	76:24	64:36
10 m*M* ammonium acetate/acetonitrile ratio (v/v); column temperature = 35°C	74:26	66:34
pH of 10 m*M* ammonium acetate; column temperature = 30°C	4.7	5.1

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Goldenseal Extract Analysis

To ensure that our method was suitable for analysis of actual goldenseal extracts, goldenseal root powder was extracted with hexane/chloroform/ethanol (1:1:4, v/v) and the resulting residue was analyzed. The results indicate the presence of 0.63% palmatine, 12.20% berberine, 8.35% hydrastine, and 0.36% canadine by weight in the extract. These values indicate weight percentages of 0.10% palmatine, 1.90% berberine, 1.30% hydrastine, and 0.06% canadine in the root powder.

Hydrastinine was not fully resolved from other components present in the residue; hence, it was not quantitated. The corresponding chromatogram for this analysis is shown in Figure 1.

REFERENCES

1. *The Complete German Commission E Monographs Therapeutic Guide to Herbal Medicines*, Blumenthal, M., Ed.; American Botanical Council: Austin, TX, 1998; 8–12.
2. *The Merck Index*, 12th Ed.; Budavari, S., Ed.; Merck & Co., Inc.: Whitehouse Station, NJ, 1996; 816.
3. Nijland, M.M. Pharm. Weekblad. **1961**, *96*, 640–641.
4. *HPLC Columns for Nutraceutical Analysis*, Phenomenex: Technical Bulletin; 1998.
5. Hermes, R.E.; Valdez, L.S. HPLC Method for the Determination of Alkaloids in Extracts of Goldenseal, [LA-UR-96-974] Analytical Chemistry Poster [ACS 211 (1–2), 1996 ANYL 77]; American Chemical Society Meeting, New Orleans, LA, March 1996.
6. Lin, S.-J.; Tseng, H.-H.; Wen, K.-C.; Suen, T.-T. J. Chromatog. A **1996**, *730*, 17–23.

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7. Sheu, S.-J.; Li, K.-L. J. High Resolut. Chromatogr. **1996**, *21*, 569–573.
8. Purification of *Coptidis rhizoma.* In *LC Application Note No. E16*; MetaChem Technologies, Inc.: Torrance, CA. Facsimile Received December 8, 1998.
9. Berberine and Coptisine. MetaChem Technologies, Inc.: Torrance, CA. Facsimile Received December 2, 1998.
10. Chen, Y.R.; Wen, K.C.; Her, G.R. J. Chromatog. A **2000**, *866*, 273–280.
11. *The United States Pharmacopoeia*, 24th Ed.; The United States Pharmacopeial Convention, Inc.: Rockville, MD, 1999; 1922–1924.

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