JPP Journal of Pharmacy and Pharmacology

JPP 2002, 54: 435–439

Received July 10, 2001
Accepted October 15, 2001
ISSN 0022-3573

A validated high performance liquid chromatographic method for the analysis of Goldenseal

Wenkui Li and John F. Fitzloff

Abstract

Goldenseal (*Hydrastis canadensis* L.) has emerged as one of the top ten herbal supplements on the worldwide market. A rapid, simple and validated high performance liquid chromatographic method, with photodiode array detection, has been developed for the analysis of commercial Goldenseal products. Samples were treated by sonication with acidified methanol/water. The method was validated for LOD, LOQ, linearity, reproducibility and recovery with good results.

Introduction

Herbal therapies have been used worldwide throughout history, but have recently undergone a renaissance. The consumption of dietary supplements has increased dramatically in recent years with domestic sales in the US of over $14 billion, and one in three Americans using herbal medication in 1999 (Mar & Bent 1999; Smith 2000). Goldenseal is one of the top ten products among these dietary supplements (Mar & Bent 1999).

Goldenseal is the dried roots and rhizomes of a native North American plant, *Hydrastis canadensis* L. (Ranunculaceae). It was originally used by American Indians as an antiseptic. Currently, it is used for the treatment of upper respiratory tract infections, often in combination with Echinacea. Chemically, Goldenseal mainly contains isoquinoline alkaloids, hydrastine, berberine, hydrastinine, tetrahydroberberine and canadine (Messana et al 1980). These alkaloids are considered to contribute to the biological activity of Goldenseal (Palmery et al 1993, 1996; Cometa et al 1996; Baldazzi et al 1998; Abdel-Haq et al 2000). The most common commercial preparations of Goldenseal are powdered roots and powdered extract. The standardization of the products is usually based on the content of hydrastine and berberine, with not less than 5% hydrastine and not less than 10% total alkaloids in powdered extract, or 2.0% hydrastine and 2.5% berberine in powdered dried roots and rhizomes (Pharmacopeial Forum 2001).

Although the interest in Goldenseal products has increased—its domestic market share accounts for 6% of all herbal products in the US today (Mar & Bent 1999)—there are only a few methods available for the quality assurance/control of Goldenseal. The published chromatographic methods for the analysis of Goldenseal include thin-layer chromatography (TLC) (Datta et al 1971), paper chromatography (PC) (Wisniewski & Gorta 1966) and high performance liquid chromatography (HPLC) (Wisniewski & Gorta 1966, 1968; Leone et al 1996; Govindan & Govindan 2000). The HPLC method has advantages over TLC and

Program for Collaborative Research in the Pharmaceutical Sciences (PCRPS, m/c 877), Functional Food for Health (FFH) Core Analytical Laboratory, and Department of Medicinal Chemistry and Pharmacognosy (m/c 781), College of Pharmacy, University of Illinois at Chicago, 833 South Wood Street, Chicago, IL 60612, USA

Wenkui Li and John F. Fitzloff

Correspondence: John F. Fitzloff, Program for Collaborative Research in the Pharmaceutical Sciences, Functional Food for Health Core Analytical Laboratory, and Department of Medicinal Chemistry and Pharmacognosy, College of Pharmacy, University of Illinois at Chicago, 833 South Wood Street, Chicago, IL 60612, USA. E-mail: fitzloff@uic.edu

PC with regard to sensitivity and selectivity. Unfortunately, none of the published HPLC methods have been fully validated in terms of LOD, LOQ, linearity, reproducibility and recovery. No details of the work-up procedure and extraction efficiencies have been given and so the work cannot be reproduced.

The objective of the present study was to develop and validate a new HPLC method with photodiode array (PDA) detection for the analysis of Goldenseal products. The extraction efficiency was also further explored.

Materials and Methods

Chemicals

All organic solvents and chemicals were of HPLC grade from Fisher Scientific Co. (Fair Lawn, NJ). Deionized water was obtained with an in-house Nano-Pure water system (Barnstead, Newton, MA).

Materials

Commercial Goldenseal and Echinacea–Goldenseal capsules were obtained from local pharmacies (Chicago, IL). The products claimed to contain 80 mg Goldenseal root powder in each capsule. To protect the manufacturers' identities, the products are labelled A and B.

Preparation of standards

The standards (hydrastine·HCl and berberine·HCl) were purchased from ChromaDex Co., (Laguna Hills, CA) and dissolved in methanol. Standard curves were obtained with concentrations over the range of 10–200 μg mL^{-1} in seven increments. Three sets of quality controls (19.96, 79.86 and 159.7 μg mL^{-1} for hydrastine; 20.08, 80.35 and 160.7 μg mL^{-1} for berberine) were prepared for hydrastine and berberine.

Sample extraction

Method 1

This method was modified from that of Leone et al (1996) and Messana et al (1980). The powder in one Goldenseal capsule was exactly weighed into a 20-mL PTFE-capped sample vial and extracted with 15 mL 90% methanol by sonication at room temperature for 60 min, and then let stand overnight. The resulting mixture was filtered through a filter paper (Whatman no. 1) into a 250-mL round-bottom flask and the residue was returned to the vial. Another 15 mL 90% methanol was added and the mixture was sonicated at room temperature for 30 min. The extract was filtered into the same flask and the residue was washed with methanol (3 × 15 mL) while on the filter. The combined extracts were evaporated to dryness, under vacuum, at 45–50°C. The residue was re-dissolved with methanol (4 × 2 mL), transferred to a 10-mL volumetric flask and made up to the volume with methanol. The solution was filtered through a 0.2-μm membrane filter just before HPLC analysis.

Method 2

This method was modified from that of Govindan & Govindan (2000). The powder in one Goldenseal capsule was exactly weighed into a 20-mL PTFE-capped sample vial and extracted with 15 mL 90% methanol (containing 1% acetic acid) by sonication at room temperature for 60 min, and then let stand overnight. The resulting mixture was filtered through a filter paper (Whatman no. 1) into a 250-mL round-bottom flask, and the residue was returned to the vial. Another 15 mL 90% methanol (containing 1% acetic acid) was added and the mixture was sonicated at room temperature for 30 min. The extract was filtered into the same flask and the residue was washed with methanol (3 × 15 mL) while on the filter. The combined extracts were evaporated to dryness, under vacuum, at 45–50°C. The residue was re-dissolved with methanol (4 × 2 mL), transferred to a 10-mL volumetric flask and made up to the volume with methanol. The solution was filtered through a 0.2-μm membrane filter just before HPLC analysis.

Reproducibility

The precision and accuracy of the method were assessed by within and between run validations. The variation was evaluated by injecting three sets of controls on three separate days. By substituting the peak area into the calibration curve equation from the same run, the measured concentrations were obtained. The coefficient of variance (RSD%) was calculated by comparing the measured concentrations. The relative errors (RE%) were obtained by comparing calculated and theoretical concentrations.

Recovery

Goldenseal powder (3.0 g) was extracted in a 50-mL flask with 30 mL 90% methanol (containing 1% acetic acid) by sonication for 60 min. After filtration, the

residue was returned to the same flask and extracted with 30 mL fresh 90% methanol (containing 1% acetic acid) by sonication for 60 min. After filtration, the above extraction procedure was repeated until no peaks were detected in the filtrate by HPLC-PDA as described below. The residue was dried before use. A portion of dried residue powder (0.5 g) was accurately weighed into a 20-mL PTFE-capped sample vial. To the vial, 1 mL of standard recovery working solution (containing berberine and hydrastine at three different concentrations: 40, 120 and 200 μg mL^{-1}) and 15 mL 90% methanol (containing 1% acetic acid) were added. The recovery sample was prepared following the method described in method 2. A blank recovery sample was prepared and analysed for the comparison.

HPLC-PDA analysis

All samples were analysed on a Waters Alliance 2690 high performance liquid chromatograph equipped with photodiode array detector, in-line degasser, column temperature controller and autosampler. Samples were eluted by gradient from a Supelco Discovery C-18 column (250 mm, 4.6 mm i.d., 5 μm particle size, lot no. 24855-08, bonded phase lot no. 3651, silica lot no. pS 183) maintained at 20°C. The mobile phase consisted of water (solvent A; containing 10% acetonitrile and 0.1% TFA) and acetonitrile (solvent B; containing 0.1% TFA), with solvent A varying from 90 to 40% over 20 min. The solvent flow rate was 1.0 mL min^{-1}. The injection volume was 10 μL for all the samples tested. The signal was monitored at 225 nm. Data collection and integration were performed using Waters Millennium software revision 32.

Results and Discussion

PDA detection

The UV wavelength for the detection of hydrastine and berberine were formerly reported over the range 280–290 nm (Leone et al 1996; Govindan & Govindan 2000). However, there was a concern that hydrastine may have weak UV absorption over this wavelength range. Figure 1 shows the PDA spectra of hydrastine and berberine over the range 200–400 nm. Obviously, both hydrastine and berberine have strong absorption at 220–240 nm. To simplify the computation of the sample chromatograms, all peaks using the PDA were integrated at 225 nm.

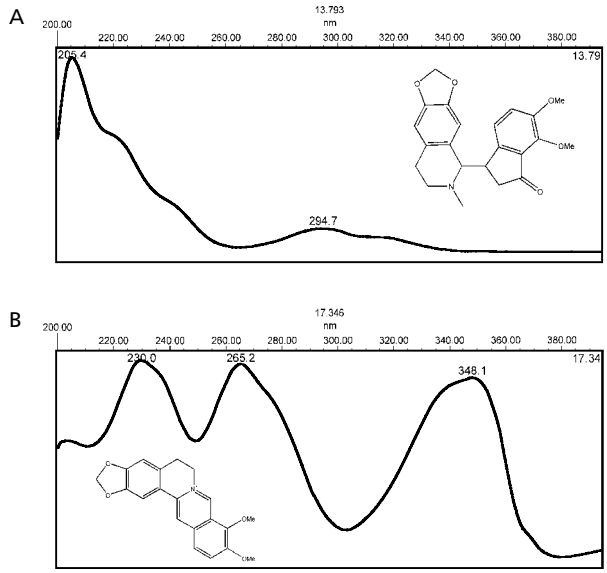

Figure 1 Photodiode array (PDA) spectra (and structures as insets) of hydrastine (A) and berberine (B) over the wavelength range 200–400 nm.

Comparison of extraction methods and extraction efficiency

Several methods have been proposed for the extraction of alkaloids in Goldenseal, including sonicating a 0.5-g sample with 2 mL 70% ethanol for 30 min, soaking a 0.5-g sample with 70% ethanol overnight and sonicating the sample with a solvent mixture (75% water (containing 5% acetic acid and 100 mM NH_4OAc), 5% methanol and 20% acetonitrile) for 60 min (Govindan & Govindan 2000), or extracting the alkaloids with ethanol (1:5, w/v) (Leone et al 1996). In the current assay, two modified extraction methods were compared by analysing replicate samples with the same lot number for extraction efficiency. As shown in Table 1, there was no significant difference in the extraction efficiency of hydrastine between Methods 1 and 2. However, the extraction efficiency of berberine using Method 2 was

Table 1 Comparison of the extraction methods.

Extraction method	Levels of alkaloids in sample (%, w/w)	
	Hydrastine (%)	Berberine (%)
Method 1	0.4667±0.004	0.7099±0.007
Method 2	0.4618±0.009	0.7619±0.015

Data are means±s.d. of three replicates.

Table 2 Reproducibility of the HPLC method.

Constituent	Spiked concn (μg mL^{-1})	Day 1			Day 2			Day 3		
		Measured concn (μg mL^{-1}) (mean±s.d., n = 3)	RSD (%)	RE (%)	Measured concn (μg mL^{-1}) (mean±s.d., n = 3)	RSD (%)	RE (%)	Measured concn (μg mL^{-1}) (mean±s.d., n = 3)	RSD (%)	RE (%)
Hydrastine	19.96	20.61±0.10	0.47	3.23	19.77±0.05	0.27	−0.97	19.79±0.50	2.55	−0.88
	79.86	73.93±0.33	0.45	3.06	76.86±0.34	0.45	−3.75	76.75±0.18	0.24	−3.89
	159.7	161.8±0.83	0.51	−7.61	161.8±0.93	0.58	0.70	160.7±1.32	0.82	0.60
Berberine	20.08	20.70±0.10	0.50	3.06	19.84±0.05	0.26	−1.23	20.07±0.54	2.71	−0.09
	80.35	74.23±0.23	0.32	−7.61	77.06±0.42	0.55	−4.10	77.07±0.28	0.36	−4.09
	160.7	162.6±0.59	0.36	1.15	162.0±0.31	0.19	0.82	161.3±1.37	0.85	0.34

greater than that of Method 1 because of the low pH value of the extracting solvent. Thus, Method 2 was chosen for the sample analysis. Further investigation revealed that greater than 93% of hydrastine and berberine in the Goldenseal samples could be extracted in the first step of sonication, and the remaining hydrastine and berberine could be extracted in the second step of sonication.

Method validation

The method was validated for linearity, sensitivity, reproducibility and recovery. The linearity of the standard curves was studied for hydrastine and berberine. The linearity is expressed in terms of the correlation coefficient (r^2). The correlation coefficient was better than 0.999 for hydrastine and berberine.

Using the statistical method proposed by Vial & Jardy (1999), the LOD for hydrastine and berberine was determined to be 1 ng (0.1 μg mL^{-1}) on the column (S/N = 3). The LOQ was found to be 4 ng (0.4 μg mL^{-1}) on the column (S/N = 10).

The reproducibility of the method was assessed by within and between run validations. The variation was evaluated by injecting three sets of controls on three consecutive days. By substituting the peak area into the calibration curve equation from the same run the measured concentrations were obtained. By comparing calculated and theoretical concentrations, the relative errors (RE%) were obtained. The coefficient of variance (RSD%) was calculated by comparing the measured concentrations. As shown in the Table 2, the RSD% and the RE% were found to be less than 2.71 and 7.62%, respectively.

Three sets of recovery samples were analysed as described above. A blank recovery sample (without adding standards) was prepared and analysed for comparison. The average recovery was observed between 98.25 and 104.20% for hydrastine and berberine, respectively (Table 3).

Table 3 Recovery of the HPLC method.

Analyte	Concn added (μg mL^{-1})	Recovery (μg mL^{-1})	RE (%)
Hydrastine	40	41.45±0.06	103.6
	120	117.90±0.41	98.25
	200	202.12±1.54	101.06
Berberine	40	41.70±0.17	104.2
	120	118.86±0.64	99.05
	200	204.07±1.38	102.03

Sample analysis

A typical HPLC chromatogram is shown in Figure 2. Two sets of samples were analysed according to the method described above. The average content of hydra-

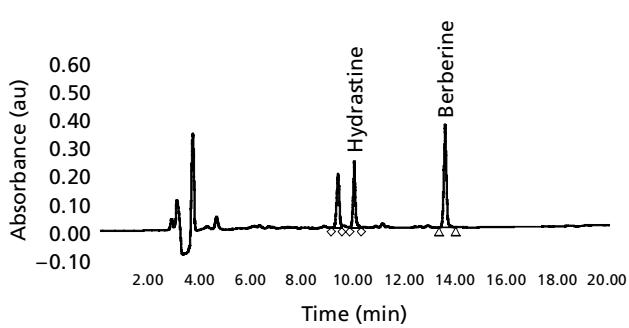

Figure 2 Typical HPLC chromatogram of a Goldenseal sample.

stine and berberine was found to be 0.45 and 0.68% (w/w) and 0.46 and 0.70% (w/w) for products A and B, respectively. This was equivalent to 2.25 and 3.4%, and 2.3 and 3.5% of hydrastine and berberine, respectively, in 80 mg of plant material. Under the current conditions, only the peaks of berberine, hydrastine and their analogues were observed in the chromatogram. This was mainly owing to the selectivity of the extraction method and chromophoric properties of the compounds tested. Obviously, the compound with a retention time of 9.3 min has a high content in the sample extract (Figure 2). It was not quantified in the current assay because of the shortage of authentic standard, although it showed similar PDA spectrum to that of hydrastine. The isolation and structural determination of the compound is underway.

References

Abdel-Haq, H., Cometa, M. F., Palmery, M., Leone, M. G., Silvestrini, B., Saso, L. (2000) Relaxant effects of *Hydrastis canadensis* L. and its major alkaloids on guinea pig isolated trachea. *Pharmacol. Toxicol.* **87**: 218–222

Baldazzi, C., Leone, M. G., Casini, M. L., Tita, B. (1998) Effects of the major alkaloids of *Hydrastis canadensis* L., berberine, on rabbit prostate strips. *Phytother. Res.* **12**: 589–591

Cometa, M. F., Galeffi, C., Palmery, M. (1996) Acute effects of alkaloids from *Hydrastis canadensis* L. on guinea pig ileum: structure–activity relationships. *Phytother. Res.* **10** (Suppl. 1): S56–S58

Datta, D., Bose, P. C., Ghosh, D. (1971) Thin layer chromatography and UV spectrophotometry of alcoholic extracts of *Hydrastis canadensis*. *Planta Med.* **19**: 258–263

Govindan, M., Govindan, G. (2000) A convenient method for the determination of the quality of Goldenseal. *Fitoterpia* **71**: 232–235

Leone, M. G., Cometa, M. F., Palmery, M., Saso, L. (1996) HPLC determination of the major alkaloids extracted from *Hydrastis canadensis*. *Phytother. Res.* **10**: S45–S46

Mar, C., Bent, S. (1999) An evidence-based review of the 10 most commonly used herbs. *West J. Med.* **171**: 168–171

Messana, I., La Bua, R., Galeffi, C. (1980) The alkaloids of *Hydrastis canadensis* L. (Ranunculaceae). Two new alkaloids: hydrastidine and isohydrastidine. *Gazz. Chim. Ital.* **110**: 539–543

Palmery, M., Leone, M. G., Pimpinella, G., Romanelli, L. (1993) Effects of *Hydrastis canadensis* L. and the two major alkaloids berberine and hydrastine on rabbit aorta. *Pharmacol. Res.* **27** (Suppl.): 73–74

Palmery, M., Cometa, M. F., Leone, M. G. (1996) Further studies on the adrenolytic activity of the major alkaloids from *Hydratis canadensis* L. on isolated rabbit aorta. *Phytother. Res.* **10**: S47–S49

Pharmacopeial Forum (2001) **26**: 944–948; **27**: 2258–2259

Smith, I. K. (2000) Ginseng surprise. *Time* (31 July 2001), p. 68

Vial, J., Jardy, A. (1999) Experimental comparison of the different approaches to estimate LOD and LOQ of an HPLC method. *Anal. Chem.* **71**: 2672–2677

Wisniewski, W., Gorta, T. (1966) Paper-chromatographic separation of berberine, hydrastine, and hydratinine in liquid extracts and rhizomes of *Hydrastis canadensis*. *Acta Pol. Pharm.* **23**: 455–458

Wisniewski, W., Gorta, T. (1968) Chromatographic–fluorimetric method for microdetermination of hydrastine in liquid extracts and rhizomes of *Hydrastis canadensis*. *Acta Pol. Pharm.* **25**: 427–433