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Narrowbore high performance liquid chromatography of berberine and palmatine in crude drugs and pharmaceuticals with ion-pair extraction using cobalt thiocyanate reagent

Hye Suk Lee [a,*], Yoo Eun Eom [a], Dong Ok Eom [b]

[a] *College of Pharmacy and Medicinal Resources Research Center, Wonkwang University, Iksan, 570-749, South Korea*
[b] *College of Pharmacy, Woosuk University, Jeonju, 565-800, South Korea*

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Abstract

For the simultaneous determination of berberine and palmatine from Phellodendri Cortex, Coptidis Rhizoma and pharmaceuticals, a new narrowbore HPLC method was developed with a simple and selective sample clean-up using cobalt thiocyanate reagent. Samples were sonicated with 2% hydrochloric acid for 30 min and protoberberine-type alkaloids in the resulting mixture were extracted with cobalt thiocyanate reagent and dichloroethane. The aliquot of dichloroethane layer was evaporated and the residue was dissolved for HPLC analysis. The recoveries of berberine and palmatine from Phellodendri Cortex, and Coptidis Rhizoma were better than 90%. Calibration curves for berberine and palmatine were linear over the concentration range of 0.1–50 μg/ml. Limits of quantitation for berberine and palmatine were 0.5 ng. This sample preparation process can be used for the identification of protoberberine-type alkaloids from crude drugs and oriental pharmaceutical preparations.

Keywords: Berberine; Palmatine; Cobalt thiocyanate reagent; Ion-pair extraction; Narrowbore reversed-phase chromatography

1. Introduction

Coptidis Rhizoma and Phellodendri Cortex are widely used as antibacterial, anti-inflammatory, and antiphlogistic agents in traditional Chinese medicine. Their major chemical constituents are protoberberine-type alkaloids such as berberine and palmatine.

Several methods for the determination of protoberberine-type alkaloids have been reported: column chromatography [1–4], spectrophotometry [5–7], thin-layer chromatography (TLC) [8–10], and high performance liquid chromatography (HPLC) [11–13]. In spectrophotometric methods, total protoberberine-type alkaloids were determined by ion-pair extraction with tetrabromophenolphthalein ethyl ester [5], bromocresol green [6], and bromophenol blue and quinine [7]. In chromatographic methods [1–4,8–13], Soxhlet extrac-

* Corresponding author. Tel.: +82-653-8506817; fax: +82-653-8507309.
E-mail address: hslee@wonnms.wonkwang.ac.kr (H.S. Lee)

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tion, ultrasonication or solvent extraction with methanol or methanol–hydrochloric acid mixture were used as the sample clean-up procedure.

Cobalt thiocyanate reagent has been used for the identification and/or assay of amodiaquine, bethanidine, and poldine in British Pharmacopeia. Arylonium ion formed water-insoluble arylonium–cobalt complex in the presence of cobalt thiocyanate ion ($[Co(SCN)_4]^{2-}$) and those complexes were soluble in organic solvents such as chloroform, dichloromethane and dichloroethane [14]. We identified that protoberberine-type alkaloids yielded cobalt complexes on the addition of cobalt thiocyanate reagent, and therefore, cobalt thiocyanate reagent can be used for the selective extraction of protoberberine-type alkaloids from complex samples such as crude drugs and pharmaceuticals.

This study describes a narrowbore HPLC method with a selective ion-pair extraction for the determination of berberine and palmatine in Coptidis Rhizoma, Phellodendri Cortex and pharmaceuticals.

2. Experimental

2.1. Materials and reagents

Berberine hydrochloride, palmatine hydrochloride and sodium octanesulfonate were obtained from Sigma (St Louis, MO). All other reagents were of HPLC grade.

To prepare cobalt thiocyanate reagent, cobaltous nitrate (36.59 g/l) and ammonium thiocyanate (239.78 g/l) were dissolved in distilled water, and then, potassium phthalate (10.26 g/l) and sodium hydroxide (1.6 g/l) were added to adjust the pH.

2.2. Chromatographic system

The HPLC system consisted of a SpectraSystem P4000 pump (Thermo Separation Products, CA), a Rheodyne 7125 injector (Cotati, CA), and a SpectraSystem UV3000 detector with a 6-μl microbore cell. Data handling was performed by a PC1000 software program.

The analytical column was a Capcell Pak UG 120 column (250 × 2 mm i.d., Shiseido, Tokyo, Japan) equipped with a Capcell Pak UG 120 guard column (10 × 2 mm i.d., Shiseido). The mobile phase was acetonitrile–phosphate buffer (50 mM, pH 4.5) containing sodium octanesulfonate (10 mM) (34:66) and the flow rate was 0.2 ml/min. Detection wavelength was 254 nm and column temperature was 30°C.

2.3. Sample preparation

Phellodendri Cortex (100 mg), Coptidis Rhizoma (20 mg) or pharmaceuticals were extracted with 2% hydrochloric acid (10 ml) by ultrasonicating for 30 min at 40°C, and cobalt thiocyanate reagent (2 ml) and dichloroethane (10 ml) were added. The mixture was shaken at 280 rev./min for 10 min and centrifuged at 2500 rev./min for 10 min. The aliquot (0.1 ml) of the organic layer was evaporated to dryness under the stream of nitrogen gas and the residue was dissolved in methanol (2 ml) to destroy the complex. The aliquot (5 μl) was injected onto HPLC.

2.4. Method validation

For the recovery test, berberine and palmatine were added to Phellodendri Cortex and Coptidis Rhizoma, in which their contents had already been determined by HPLC. Limit of quantitation (LOQ) was evaluated at a signal-to-noise ratio of 5:1.

3. Results and discussion

3.1. Chromatography

Ion-pair HPLC methods [11,12] and reversed-phase HPLC [13] were reported for the simultaneous determination of protoberberine-type alkaloids. Sodium dodecyl sulfate was used as an ion-pair reagent at a concentration range of 17–50 mM at pH 2.2.

In this study, a narrowbore HPLC method was chosen due to higher column efficiency, increased detectability and lower solvent consumption. Cap-

cell Pak UG 120 column, pH-stable octadecyl silica column, was used as the stationary phase. Effects of pH and the concentration of sodium octanesulfonate in mobile phase on capacity factors of berberine and palmatine were evaluated to achieve satisfactory resolution; 34% acetonitrile in 0.05 M phosphate buffer (pH 4.5) containing 10 mM sodium octanesulfonate was found to be the best (Fig. 1).

3.2. Sample preparation

Protoberberine-type alkaloids from Phellodendri Cortex and Coptidis Rhizoma were extracted by Soxhlet extraction, ultrasonication or shaking with methanol or methanol–hydrochloric acid mixture (100:1) [1–13]. In this study, methanol was inappropriate as an extraction solvent because it hindered the complex formation of protoberberine-type alkaloids with cobalt thiocyanate. Ultrasonication of the samples with 2% hydrochloric acid for 30 min was enough for the extraction of berberine and palmatine from crude drugs and pharmaceuticals (Table 2).

Protoberberine-type alkaloids formed green protoberberine–cobalt complexes which were freely soluble in dichloromethane and dichloroethane compared to chloroform, ethyl acetate and benzene and had a specific absorbance at 625 nm. Therefore, dichloroethane was used as extraction solvent of protoberberine–cobalt complexes because of low volatility compared to dichloromethane. Addition of cobalt thiocyanate reagent and dichloroethane to 2% hydrochloric acid extracts of Coptidis Rhizoma, Phellodendri Cortex and pharmaceuticals resulted in the selective extraction of protoberberine-type alkaloids as green protoberberine–cobalt complexes into the dichloroethane layer. From these results, cobalt thiocyanate reagent can be used for the identification of protoberberines from pharmaceuticals.

3.3. Method validation

Mean absolute recoveries of berberine from Phellodendri Cortex and Coptidis Rhizoma were 95.8 ± 2.7 and $91.6 \pm 1.7\%$, respectively (Table 1). The mean absolute recoveries of palmatine from Phellodendri Cortex and Coptidis Rhizoma were 92.3 ± 2.1 and $95.4 \pm 1.9\%$, respectively (Table 1). The coefficient of variation (C.V.) of the assay for berberine and palmatine varied from 1.6 to 6.1%.

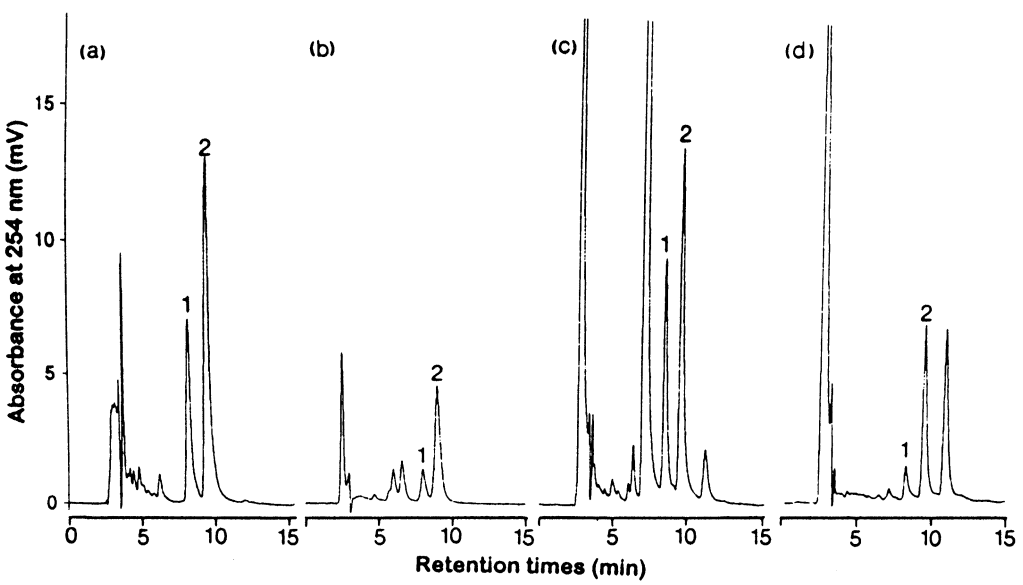

Fig. 1. HPLC chromatograms of berberine and palmatine in crude drugs and pharmaceuticals: (a) Phellodendri Cortex; (b) Coptidis Rhizoma; (c) a commercial syrup containing seven crude drugs; (d) a commercial tablet. Peaks: 1, palmatine; 2, berberine.

Table 1
Recovery of berberine and palmatine from Coptidis Rhizoma and Phellodendri Cortex ($n = 8$)

Protoberberines	Amount added (mg)	Amount found* (mean ± S.D., mg)	
		Coptidis Rhizoma (%)	Phellodendri Cortex (%)
Berberine	0.3	0.28 ± 0.02 (93.3)	0.28 ± 0.01 (93.3)
	0.6	0.55 ± 0.02 (91.7)	0.58 ± 0.03 (96.7)
	1.2	1.07 ± 0.07 (89.2)	1.19 ± 0.05 (99.2)
	2.4	2.21 ± 0.03 (92.1)	2.25 ± 0.07 (93.8)
	Average	91.6 ± 1.7	95.8 ± 2.7
Palmatine	0.3	0.27 ± 0.02 (90.0)	0.27 ± 0.01 (90.0)
	0.6	0.59 ± 0.04 (98.3)	0.55 ± 0.02 (91.7)
	1.2	1.15 ± 0.05 (95.8)	1.11 ± 0.03 (92.5)
	2.4	2.34 ± 0.03 (97.5)	2.28 ± 0.03 (95.0)
	Average	95.4 ± 1.9	92.3 ± 2.1

* Corrected for the original berberine and palmatine present in Coptidis Rhizoma and Phellodendri Cortex sample used.

Calibration curves for berberine and palmatine were linear with correlation coefficients of 0.999 over the concentration range of 0.1–50 μg/ml. LOQ for berberine and palmatine were 0.5 ng.

3.4. Analysis of various pharmaceuticals

This method was successfully applied to the identification and determination of berberine and palmatine from commercial pharmaceutical samples such as syrup and tablet (Table 2). Protoberberine-type alkaloids were qualitatively identified from the presence of the green dichloroethane layer in the sample preparation. As shown in Fig. 1c,d, other constituents from syrup and tablet samples could be extracted by dichloroethane in place of complex formation.

4. Conclusions

For the determination of berberine and palmatine from Coptidis Rhizoma, Phellodendri Cortex and pharmaceuticals, the new narrowbore HPLC method was developed using a selective, rapid, and simple ion-pair extraction. The samples were ultrasonicated with hydrochloric acid, and protoberberine-type alkaloids were selectively extracted as complexes by cobalt thiocyanate reagent and dichloroethane. Quantitative as well as qualitative analysis of berberine and palmatine from Coptidis Rhizoma, Phellodendri Cortex and pharmaceuticals were successfully performed using ion-pair extraction with cobalt thiocyanate reagent.

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Table 2
Determination of berberine and palmatine in crude drugs and pharmaceuticals[a]

Sample	Berberine (%)	Palmatine (%)
Phellodendri Cortex	0.52 ± 0.03	0.29 ± 0.02
Coptidis Rhizoma	5.0 ± 0.3	2.1 ± 0.1
Preparation A (syrup)	1.18 ± 0.07	0.53 ± 0.03
Preparation B (tablet)	8.0 ± 0.1	0.45 ± 0.02

[a] Values are mean ± S.D., $n = 5$. Preparation A (in 100 ml): Cinnami Cortex, 700 mg; Coptidis Rhizoma, 300 mg; Gambir, 3340 mg; Ginseng Radix Alba, 300 mg; Glycyrrhizae Radix extract, 1000 mg; Phellodendri Cortex, 2000 mg; Scutellariae Radix, 300 mg; methyl-*p*-hydroxybenzoate, 50 mg; propyl-*p*-hydroxybenzoate, 50 mg. Preparation B (one tablet): Berberine chloride, 22.5 mg; bismuth subnitrate, 225.0 mg; furazolidone, 45.0 mg; Scopolia extract, 10.5 mg.

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