Application of Ion-Pair High Performance Liquid Chromatography for Analysis of Hyoscyamine and Scopolamine in Solanaceous Crude Drugs

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A new ion-pair high-performance liquid chromatographic method for the analysis of hyoscyamine and scopolamine in various kinds of crude drugs derived from solanaceous plants was evaluated using sodium dodecyl sulfate as a counter ion. A reversed-phase chromatographic system consisting of a chemically bonded ODS silica gel column with phosphate buffer (pH 2.5)–acetonitrile (65:35) containing 17.5 mM sodium dodecyl sulfate as the mobile phase was used. Hyoscyamine and scopolamine in crude drugs derived from Scopolia, Atropa, Hyoscyamus and Datura species were separated from other compounds in the crude drugs and determined within 20 min after direct injection of the solution extracted with the mobile phase. The results for various kinds of samples are presented.

Keywords ion-pair high-performance liquid chromatography; scopolamine; hyoscyamine; atropine; *Scopolia japonica*; *Atropa belladonna*; *Hyoscyamus niger*; *Datura metel*

The crude drugs derived from solanaceous plants, such as *Scopolia japonica*, *Atropa belladonna*, *Hyoscyamus niger* and *Datura metel*, contain tropane alkaloids and are used as anodynes and anticonvulsants. Among these tropane alkaloids, hyoscyamine and scopolamine are important compounds for evaluating the potency of the crude drugs from the pharmacological point of view. The analysis of hyoscyamine and scopolamine in solanaceous crude drugs and their extract has been examined by means of paper partition chromatography (PPC),[1] thin layer chromatography (TLC),[2-4] gas liquid chromatography (GLC)[5,6] and high performance liquid chromatography (HPLC).[7-16] HPLC is useful for the analysis of tropane alkaloids from the viewpoints of resolution and sensitivity. Although several HPLC methods have been applied to solanaceous crude drugs, they require time-consuming pretreatment, for example partition between alkaline ammonia solution and chloroform or diethyl ether, because unknown impurities interfere with scopolamine and/or hyoscyamine on the chromatogram. There are two mobile phase systems for HPLC which can be used to determine scopolamine and hyoscyamine after direct injection of the solution extracted by methanol or a mixture of water and methanol. Anetai and Yamagishi[13,14] applied an HPLC method which is not an ion-pair technique to analyze several kinds and parts of crude drugs and its original plants, but it is suitable only for hyoscyamine, and the scopolamine peak overlaps with peaks of impurities. Fujita[15] has developed an ion-pair method for the analysis of hyoscyamine and scopolamine in Scopolia extract. However, this method was not applicable for other solanaceous crude drugs.

Recently, sodium dodecyl sulfate (SDS) has been used as a counter-ion for the analysis of alkaloids in crude drugs, namely Coptidis Rhizoma, Phellodendri Cortex[17] and Corydalis Tuber[18] for berberine-type alkaloids, and Ephedrae Herba[19] for ephedrine alkaloids. SDS has a longer alkyl chain than the other compounds usually used for ion-pair chromatography such as pentane sulfonate and heptane sulfonate, and causes basic compounds to be more strongly retained on an ODS column by alkyl sulfate than the same chain-length alkyl sulfonate. Therefore, hyoscyamine and scopolamine should be separated from impurities other than alkaloids in the crude drugs by the long-chain alkyl sulfate in the mobile phase.

In this study, a new method of ion-pair high performance liquid chromatographic determination was applied to hyoscyamine and scopolamine in various kinds of solanaceous crude drugs using SDS as a counter ion.

Experimental

Plant Materials Commercial *Scopolia* roots, *Belladonna* leaves, *Datura* leaves, *Datura* flowers (*Datura metel*), and *Hyoscyamus* leaves and seeds were purchased in Japanese and Chinese markets. *Hyoscyamus niger* was collected at Linfen and Datong (Shanxi) and Gingyang (Gansu). *Atropa belladonna* was collected at West Beijing and Wenzhou (Zhejing).

Apparatus A Shimadzu LC-6A liquid chromatograph equipped with a Shimadzu SPD-6A ultraviolet (UV) spectrophotometer and a stainless-steel column (150 × 4 mm i.d.) packed with chemically bonded ODS silica gel (TSK gel 120A, 5 μm) (Toyo Soda) was used. The peak area was evaluated by a SIC 7000A integrator.

Reagents Atropine hydrosulfate (*dl*-hyoscyamine hydrosulfate) and scopolamine hydrobromide were purchased from Wako (Tokyo, Japan). Hexyl, octyl, decyl and tetradecyl sulfuric acid sodium salts were purchased from Merck, and SDS of biochemical grade was from Wako. Acetonitrile of chromatographic grade was used.

HPLC Conditions Phosphate buffer (1/15 M, pH 2.5)–acetonitrile (65:35) containing 17.5 mM SDS was used as the mobile phase. The column temperature was maintained at 35 °C and the flow-rate was 1.5 ml/min. The substances eluted were detected with a UV detector at 210 nm.

Assay Procedure Direct Extraction: Dry powders of the crude drugs (0.5 g) were placed in 25 ml of the mobile phase, refluxed for 30 min, cooled, centrifuged at 1600 *g* and decanted. The residue was washed twice with 10-ml portions of the mobile phase. The extracts and washings were placed in a 50-ml volumetric flask and diluted to 50 ml with the mobile phase. A 10-μl volume of this solution was injected into the HPLC system. The contents of hyoscyamine and scopolamine in the crude drugs were calculated from the relevant peak areas.

Alkaloid Fractionation: The dry powders of the crude drugs (0.5 g) were placed in 15 ml of diluted ammonia (1→6) and 6 g of sodium chloride was added. They were extracted with 20 ml of diethyl ether three times, and the diethyl ether layer was dried over anhydrous sodium sulfate and evaporated. Chloroform (5 ml) and 0.2 N sulfuric acid (5 ml) were added to the residue. After shaking, the mixture was centrifuged and 1 ml of the 0.2 N sulfuric acid layer was diluted with the mobile phase to 10 ml for use as the sample solution of the alkaloid fraction.

Calibration Graphs and Detection Limits The calibration graphs for hyoscyamine and scopolamine were obtained over the concentration range of 1.5—70.0 and 1.5—70.0 μg/ml, respectively. The corresponding regression equations were $y = 826x - 80$ ($r = 0.999$) and $y = 827x - 120$ ($r = 0.999$) and the detection limits were 10 and 10 ng, respectively, at a signal-to-noise ratio of 3:1 for the peak heights.

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Results and Discussion

HPLC Conditions Elution parameters such as the organic content of the mobile phase, the type and concentration of the counter ion, the pH and the column temperature were varied to find the optimal elution conditions with chemically bonded ODS silica gel.

Since hyoscyamine and scopolamine are alkaloids, a mobile phase containing a counter ion, such as pentane sulfonic acid salt or heptane sulfonic acid salt, has been used for the analysis. These ion-pair HPLC methods are applicable to the sample solution after pretreatment, *i.e.*, fractionation by partition between alkaline ammonia solution and chloroform or diethyl ether. However, when the sample solution extracted with some solvents was directly injected, the ion-pair mobile phase system[15] using sodium pentane sulfonate as a counter ion could separate hyoscyamine, but the scopolamine peak was overlapped by other compounds in the crude drugs. Although the phosphate buffer mobile phase system[13,14] containing triethylamine could be applicable for the separation of hyoscyamine and scopolamine from the other components in *Scopolia* root, it was inapplicable to other species. A superior counter ion was required, therefore. The length of alkyl chain of the counter ions influences the elution time of alkaloids. Sodium hexyl, octyl, decyl, dodecyl and tetradecyl sulfates were examined for the separation of tropane alkaloids from the other components in the crude drugs. Each counter ion was added to a mixture of 1/15 M phosphate buffer (pH 2.5) and acetonitrile (65 : 35) at a final concentration of 17.5 mM. As shown in Fig. 1, the retention of hyoscyamine and scopolamine increased in proportion to the length of the alkyl chain of the counter ions, and SDS allowed the best separation from the other components.

The SDS concentration in the mobile phase was varied from 0 to 35 mM. The retention of hyoscyamine and scopolamine depended on the concentration of SDS (Fig. 2).

The acetonitrile concentration was varied from 29 to 41%. Hyoscyamine and scopolamine began to separate from the other peaks at an acetonitrile concentration of 35%, and therefore, this concentration was selected for subsequent work, based on resolution and retention time.

The pH of the mobile phase affected the separation of scopolamine and the front peak. Although acidic solution (pH 1.9) to which only phosphoric acid had been added was used for this ion-pair chromatography, no separation between scopolamine and the front peak was obtained. These peaks were able to be separated at pH 2.5. A temperature of 35 °C gave the best result.

Finally, a mobile phase consisting of phosphate buffer (pH 2.5)–acetonitrile (65 : 35) containing 17.5 mM SDS al-

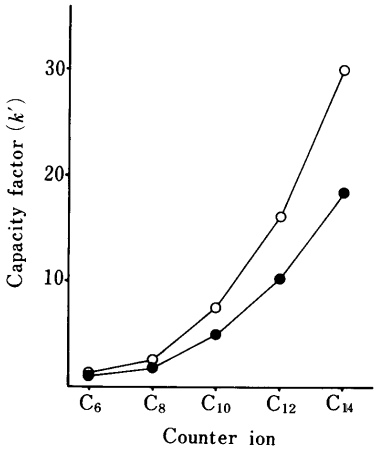

Fig. 1. Effect of Various Counter Ions on the Capacity Factor (k')

○, atropine (hyoscyamine); ●, scopolamine. Counter ion: C_6, sodium hexyl sulfate; C_8, sodium octyl sulfate; C_{10}, sodium decyl sulfate; C_{12}, SDS; C_{14}, sodium tetradecyl sulfate. Flow rate, 1.5 ml/min. Temperature, 35 °C. The mobile phase was a mixture of 1/15 M phosphate buffer (pH 2.5) and acetonitrile (65 : 35).

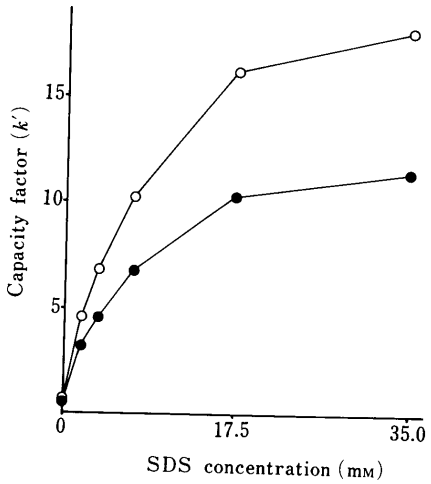

Fig. 2. Effect of SDS Concentration on the Capacity Factor (k')

Symbols and HPLC conditions, see Fig. 1.

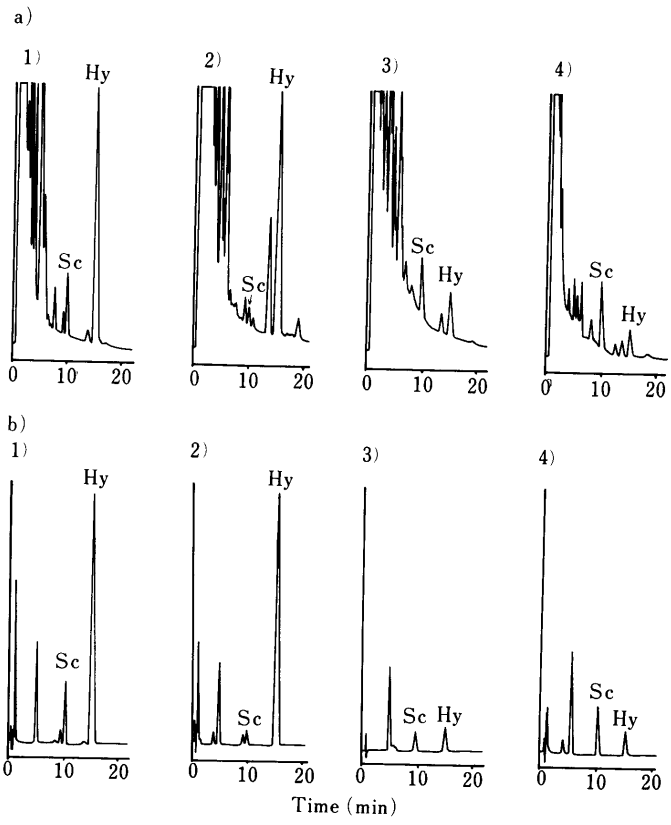

Fig. 3. Chromatograms of Solanaceous Crude Drugs

(a) Extracted by the mobile phase, (b) fractionated between alkaline ammonia solution and ether. Peaks, Hy, hyoscyamine; Sc, scopolamine. Samples: 1), *Scopolia* root (China); 2), *Atropa belladonna* (west Beijin); 3), *Hyoscyamus niger* (Linfen, Shanxi); 4), *Datura metel* (China). The mobile phase was a mixture of 1/15 M phosphate buffer (pH 2.5) and acetonitrile (65 : 35) containing 17.5 mM SDS. Other conditions, see Fig. 1.

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TABLE I. Contents of Tropane Alkaloids in Solanaceous Crude Drugs (%)

Sample	Hyoscyamine (%)	Scopolamine (%)	Total (%)
Scopolia root			
China	0.433	0.063	0.496
China	0.347	0.036	0.383
China	0.442	0.094	0.536
Korea	0.397	0.082	0.479
Korea	0.424	0.073	0.497
Korea	0.697	0.046	0.743
Korea	0.399	0.051	0.450
Korea	0.426	0.028	0.454
Korea	0.508	0.048	0.556
Russia	0.382	0.035	0.417
Russia	0.752	0.068	0.820
Atropa belladonna			
West Beijin	0.360	0.036	0.396
Wenzhou (Zhejing)	0.284	0.037	0.321
Belladonna leaf	0.403	0.054	0.457
Hyoscyamus niger			
Linfen (Shanxi)	0.063	0.065	0.128
Datong (Shanxi)	0.069	0.043	0.112
Gingyang (Gansu)	0.066	0.021	0.087
Hyoscyamus leaf	0.020	0.038	0.058
Datura meter			
China	0.103	0.262	0.365
China	0.134	0.284	0.418
Datura leaf	0.059	0.193	0.252

lowed the best separation of hyoscyamine and scopolamine from the impurities in the crude drugs.

Comparison of Chromatograms between Direct Extraction and Pre-treatment Methods Since the solution extracted by the mobile phase was injected into the column in the direct method, partition between alkaline ammonia solution and diethyl ether as a pretreatment was used for the fractionation of only the alkaloids to confirm that there are no compounds giving peaks that overlap with the hyoscyamine and scopolamine peaks. In this study, after evaporation of the diethyl ether solution the residue was dissolved in 0.2 N sulfuric acid and washed with chloroform to separate tropane alkaloids completely. Figure 3 shows the chromatograms of four kinds of solanaceous crude drugs extracted by two different methods. The hyoscyamine and scopolamine peaks appeared on both chromatograms and the analytical values of hyoscyamine and scopolamine extracted by the mobile phase were in good accordance with those obtained by the fractionation method except for the *Hyoscyamus* seed. The seed contains a lot of fatty acids so that the partition method could not extract the alkaloids completely. A peak at about 5 min is due to an impurity derived from chloroform and another peak eluted just in front of the scopolamine peak was found from the *Scopolia* and *Atropa* species (Fig. 3b). The peak in front of scopolamine is anisodamine judging from the retention time of the standard compound. *Hyoscyamus* and *Datura* species did not contain any other alkaloids.

Analytical Results Table I gives the analytical results of solanaceous crude drug samples by the ion-pair method. The contents of hyoscyamine in *Scopolia* and *Atropa* species were from 5 to 10 times higher than that scopolamine. *Datura* species contained scopolamine with larger amounts of hyoscyamine. The contents of scopolamine and hyoscyamine in *Hyoscyamus* species were both low. It is noteworthy that the contents and ratios of hyoscyamine and scopolamine showed similar values in the same species.

Conclusions

An ion-pair HPLC technique was applied to the determination of hyoscyamine and scopolamine in solanaceous crude drugs. SDS as the counter ion in the mobile phase influenced the behavior of hyoscyamine and scopolamine on the ODS column, and allowed their separation from other components in these crude drugs that interfered with scopolamine and hyoscyamine under other HPLC conditions unless time-consuming pretreatment was employed.

This method is simple and should be widely applicable for the analysis of tropane alkaloids in solanaceous crude drugs.

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