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Determination of sennoside A and B in the pharmaceutical preparation Otsuji-to using ion-pair high-performance liquid chromatography with column switching

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ABSTRACT

Ion-pair high-performance liquid chromatography with column switching was used to determine sennoside A and B in the oriental pharmaceutical preparation Otsuji-to. The fraction containing sennoside A and B eluted immediately from the pretreatment column and was transferred to the analysis column where it was separated by the ion-pair mobile phase. Sennoside A and B in Otsuji-to were determined with recoveries of 100%.

INTRODUCTION

The oriental pharmaceutical preparation Otsuji-to [1], which is used for the treatment of haemorrhoids, is prepared from six crude drugs: *Angelicae radix* (Japanese angelica root), *Bupleuri radix* (bupleurum root), *Scutellariae radix* (scutellaria root), *Glycyrrhizae radix* (glycyrrhiza), *Cimicifugae rhizoma* (cimicifuga rhizome) and *Rhei rhizoma* (rhubarb) [2]. The sennosides in *Rhei rhizoma* are well known as laxatives and the main components are sennoside A and B (Fig. 1).

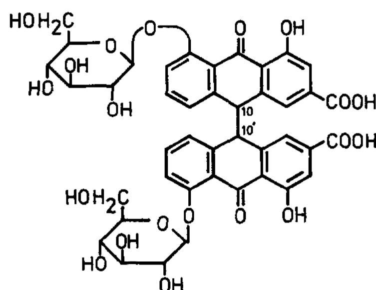

Fig. 1. Structures of sennoside A and B. Sennoside A, 10–10′ = *threo*: sennoside B, 10–10′ = *erythro*.

In work using high-performance liquid chromatography (HPLC) for the determination of natural

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products in crude drugs, sennoside A and B were determined in *Rhei rhizoma* by an ion-pair technique using tetra-*n*-heptylammonium bromide as the counter ion [3]. However, this method was not applicable to the determination of sennoside A and B in Otsuji-to because of the low concentration of *Rhei rhizoma* and interferences from the other drugs present.

However, the combination of ion-pair HPLC and column switching allowed the determination of tropane alkaloids in complex preparations without complicated pretreatment [4].

In this work, ion-pair HPLC and column switching were used for the determination of sennoside A and B in Otsuji-to.

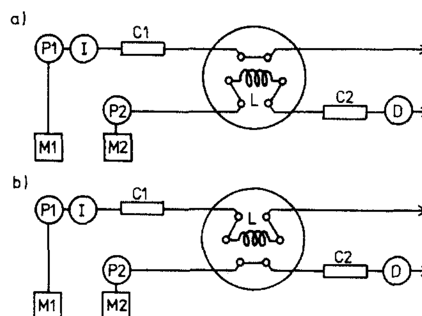

Fig. 2. Column switching system. (a) Waiting and analysis mode; (b) trapping mode. P1, P2 = pumps; C1 = pretreatment column; C2 = analysis column; D = detector; L = loop; M1 = primary mobile phase; M2 = secondary mobile phase; I = injector.

EXPERIMENTAL

Reagents

Sennoside A was purchased from Wako Pure Chemical (Osaka, Japan), sennoside B from Alps Pharmaceutical (Gifu, Japan) and tetra-*n*-heptylammonium bromide from Aldrich (Milwaukee, WI, USA). Acetonitrile of chromatographic grade was used; deionized water was further purified using a Millipore filter.

Apparatus

The Shimadzu LC-6AD system consisted of: a SIC chromatocoder 12 computing integrater; two Shimadzu LC-6AD pumps; a Shimadzu SCL-6B system controller; a Shimadzu SIL-6B autoinjector; a Shimadzu CTO-6A column oven; a sample loop switching valve; Rheodyne 7027 Shimadzu FCV-2AH; and a Shimadzu SPD-6A UV detector. The pretreatment (50 × 4 mm I.D.) and analysis (250 × 4 mm I.D.) columns were packed with chemically bonded ODS silica gel (TSKgel ODS-120A, 5 μm, Tosoh, Tokyo, Japan) by slurry-packing. The loop volume was 1 ml. This switching system is shown in Fig. 2.

HPLC conditions

A mixture of water, acetonitrile and acetic acid (680:320:1) was used as the primary mobile phase for the pretreatment column, and a mixture of pH 5.0 acetate buffer (0.1 *M*) and acetonitrile (680:320) containing 15 m*M* tetra-*n*-heptylammonium bromide was used as the secondary mobile phase for the analysis column. The column temperature were maintained at 50°C and the flow-rates of the mobile phases were 1.0 ml/min. The eluted substances were detected by UV absorption at 340 nm.

Sample preparation

Otsuji-to was prepared in this laboratory from a mixture of six crude drugs: *Angelicae radix* (5 g), *Bupleuri radix* (4 g), *Scutellariae radix* (3 g), *Glycyrrhizae radix* (3 g), *Cimicifugae rhizoma* (1.5 g) and *Rhei rhizoma* (1 g). About 340 ml of water were added to the mixture and reduced by boiling to about 170 ml. The supernatant is Otsuji-to. A blank was prepared in the same manner but without *Rhei rhizoma*.

Assay procedure

A 10-μl portion of the sample solution filtered through a membrane filter (0.45 μm) was injected into the pretreatment column of the HPLC system. The eluate fraction from 0.35 to 0.85 min was trapped in the loop and transferred to the analysis column. The sennoside A and B concentrations were calculated from the relevant peak areas.

Calibration graphs and detection limits

The calibration graphs for sennoside A and B using column switching were obtained for the concentration ranges 5.36–53.60 and 2.35–23.45 μg/ml, respectively. The corresponding regression equations were: $y = 6000x - 1200$ ($r = 0.999$) and $y = 5564x + 1531$ ($r = 0.999$).

The detection limits for sennoside A and B were 1.2 and 1.0 ng, respectively, at a signal-to-noise ratio of 3 for the peak height.

RESULTS AND DISCUSSION

Ion-pair HPLC methods have been applied to the determination of several natural ionic products in crude drugs [5–9]. Ion-pair HPLC has several advantages for such determinations, including the fact that the counter ion only affects the ionic compounds and it is possible to control the retention time by changing the counter ion and its concentration. The pH of the mobile phase also influences the peak retention time.

A simple and rapid ion-pair HPLC method [3] was developed for the determination of sennoside A and B in *Rhei rhizoma*. This method is applicable to some simple oriental pharmaceutical preparations. However, this method is not applicable to the determination of these compounds in the complex oriental pharmaceutical preparation Otsuji-to, because the concentration of *Rhei rhizoma* is low and there are interferences from other components, mainly *Scutellariae radix*. *Scutellariae radix* contains a large amount of acidic components, including baicalin and other flavone glucuronides. Sennoside A is not separated from these compounds (Fig. 3). Several HPLC methods have been studied to determine sennoside A and B in *Rhei rhizoma* [10–14]; however, except for this method, none has been reported for the determination of sennoside A and B in Otsuji-to. Column switching has frequently been used for on-line sample clean-up, the analysis of complex preparations and the determination of trace amounts of biological materials [15]. This technique is applicable to the determination of natural products in crude drugs or pharmaceutical preparations.

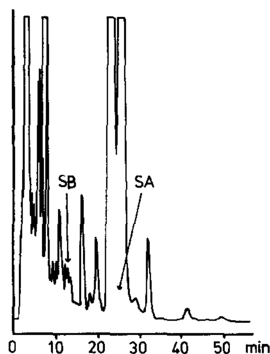

Fig. 3. Chromatogram of Otsuji-to by direct analysis. Mobile phase, pH 5.0 acetate buffer (0.1 *M*)–acetonitrile (680:320) containing 5 m*M* tetra-*n*-heptylammonium bromide; column, TSKgel ODS-120A, 5 μm, 250 × 4 mm I.D. Peaks: SA = sennoside A; SB = sennoside B.

Combinations of these two techniques have allowed the on-line determination of tropane alkaloids in complex preparations without complicated pretreatment [4]. The tropane alkaloid fraction immediately eluted with the primary mobile phase, without the counter ion, from a pretreatment column and was then transferred to an analysis column and separated by the ion-pair mobile phase. This strategy was applied to the determination of sennoside A and B, which are acidic compounds, in Otsuji-to.

HPLC conditions

At first, a mixture of pH 5.0 acetate buffer (0.1 *M*) and acetonitrile (680:320) was used as the primary mobile phase for the 50 mm long pretreatment column, and the primary mobile phase containing 5 m*M* tetra-*n*-heptylammonium bromide was used as the secondary mobile phase for the 250 mm long analysis column. Under these conditions, in the standard solution, the sennoside A and B fraction immediately eluted with the primary mobile phase and was transferred to the analysis column where it was separated by the ion-pair mobile phase. However, in the sample solution, a large amount of acidic compounds, especially baicalin and other flavone glucuronides from *Scutellariae radix*, showed a similar behaviour to that of sennoside A and B in the pretreatment column and the sennosides could not be separated from these compounds.

Ion suppression was tried for the pretreatment column. A water–acetonitrile–phosphoric acid system and a water–acetonitrile–acetic acid system were tested for use as the primary mobile phase. When water–acetonitrile–acetic acid (680:320:1) was used, sennoside A and B were eluted immediately and most of the other acidic compounds were suppressed and retained in the pretreatment column.

Under the original analysis conditions, the sennoside A and B peaks were slightly broader and sennoside B was not clearly separated from the other components after column switching with the initial

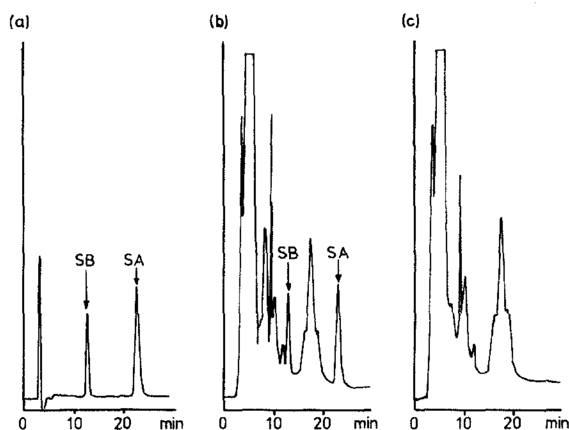

Fig. 4. Chromatograms of (a) standards, (b) Otsuji-to and (c) the blank preparation by column switching. Peaks: SA = sennoside A; SB sennoside B.

primary mobile phase. The concentration of the counter ion for the secondary mobile phase was varied to find the optimum conditions. The final concentration for the secondary mobile phase was selected as 15 m*M*.

When the fraction eluted from 0.35 to 0.85 min after injection into the pretreatment column was transferred to the analysis column, the recoveries of the sennoside A and B standards from the pretreatment column were 100.1% [$n = 5$, coefficient of variation (C.V.) = 1.1%] and 100.3% ($n = 5$, C.V. = 1.1%), respectively.

Analysis of Otsuji-to

Fig. 4 shows the chromatograms of standards, Otsuji-to and the blank preparation. The blank preparation which had no *Rhei rhizoma* present, was prepared to check the interferences at the positions of sennoside A and B on the chromatogram. Most interferences from the other crude drugs were removed by this column switching technique, and no peak appeared at the sennoside A and B positions. The concentrations of sennoside A and B in Otsuji-to prepared in this work were 20.83 μg/ml ($n = 5$, C.V. = 1.2%) and 8.36 μg/ml ($n = 5$, C.V. = 1.1%), respectively. The recoveries of sennoside A and B added to the blank preparation were 99.8% ($n = 5$, C.V. = 1.0%) and 99.5% ($n = 5$, C.V. = 1.0%), respectively.

CONCLUSIONS

The determination of sennoside A and B in Otsuji-to was achieved by a combination of ion-pair HPLC and the column switching technique without complicated pretreatment. This system seems to be applicable to the on-line determination of other ionic components in complex preparations.

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