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Journal of Chromatography A, 786 (1997) 371–376

JOURNAL OF CHROMATOGRAPHY A

Short communication

Determination of berberine and palmatine in Phellodendri Cortex using ion-pair supercritical fluid chromatography on-line coupled with ion-pair supercritical fluid extraction by on-column trapping

Keiichi Suto[a,*], Shinichi Kakinuma[a], Yuji Ito[a], Kazuhiko Sagara[a], Hideki Iwasaki[a], Hideji Itokawa[b]

[a]*OTC Product R&D Research Laboratories, Taisho Pharmaceutical Co., Ltd., 403, Yoshino-Cho 1-Chome, Omiya-Shi, Saitama 330, Japan*

[b]*Tokyo University of Pharmacy and Life Science, 1432-1 Horinouchi, Hachioji, Tokyo 192-03, Japan*

Received 24 February 1997; received in revised form 12 May 1997; accepted 22 May 1997

Abstract

An assay of berberine and palmatine in Phellodendri Cortex was established using ion-pair supercritical fluid chromatography (IP-SFC) on-line coupled with ion-pair supercritical fluid extraction (IP-SFE). The on-column method was employed to successfully perform on-line SFE–SFC. With a silica-gel column as a trapping and separation column, berberine and palmatine were extracted and focused at the column head, even if 10% methanol containing dioctyl sodium sulfosuccinate (DSS) was added to the supercritical carbon dioxide as an entrainer. After consecutive extraction and adsorption, berberine and palmatine were desorped and SFC was performed increasing the concentration of DSS methanol solution to 15%. The addition of DSS improved the efficiency of extraction of alkaloids, and the recovery of the analytes of interest was almost the same as that obtained with solvent extraction. Determination of the analytes in Phellodendri Cortex was achieved with only a few mg of crude sample. SFE was completed within ca. 10 min, and SFC was also achieved within ca. 10 min. The consecutive procedures of extraction, concentration and analysis were thus completed within ca. 20 min. Commercial Phellodendri Cortex was tested for determination of the analytes, and results confirmed that our on-line SFE–SFC method can be used for rapid convenient assay of berberine and palmatine in Phellodendri Cortex. © 1997 Elsevier Science B.V.

Keywords: Phellodendri Cortex; Supercritical fluid extraction–supercritical fluid chromatography; Pharmaceutical analysis; Berberine; Palmatine; Alkaloids; Dioctyl sulfosuccinate

1. Introduction

Supercritical fluid chromatography (SFC) can be used to separate thermally labile and high-molecular-mass samples unsuitable for GC analysis. Compared to HPLC, it offers high separation efficiencies, shorter analysis times and a wider range of possible detection methods [1].

Several features of supercritical fluid have increased its use for sample pretreatment by supercritical fluid extraction (SFE) [2].

SFC coupled with SFE achieves rapid and efficient analysis by a consecutive procedure of extraction, concentration and separation. Developments in the

*Corresponding author.

PII S0021-9673(97)00590-6

use of supercritical fluids in coupled systems were reviewed [3].

We have published a rapid and efficient method for characterization of herbal medicine using SFE and SFC [4,5].

In this report, we describe the use of the coupled SFE–SFC system for the quantitative determination of berberine and palmatine in Phellodendri Cortex.

Neutralization of charged species by ion-pair (IP) formation is a way of reducing the polarity of ionic compounds and thus increasing their solubility in supercritical carbon dioxide when polar cosolvent addition is ineffective [6,7].

Dioctyl sodium sulfosuccinate (DSS) is a surfactant, which is highly soluble in nonpolar organic solvents. We therefore tested its use as the counter-ion in this method. DSS had satisfactory solubility in supercritical carbon dioxide modified with methanol and selectivity for alkaloids. The IP-SFE method using DSS as the counter-ion was used for extraction of berberine and palmatine from Phellodendri Cortex, and efficient, selective and rapid extraction of these two chemicals was achieved.

Phellodendri Cortex is prescribed in many Japanese and Chinese traditional medicines. Berberine alkaloids (Fig. 1) are the main components of Phellodendri Cortex [8].

Analytical methods using HPLC have been reported for their determination [9,10]. However, these methods required manual extraction. Recently, SFE, SFC and coupled SFE–SFC techniques have been reported to simplify the analytical procedure [11–13]. In this report, the use of IP-SFE coupled with IP-SFC by on-column trapping is described.

In our on-line SFE–SFC, the analytes of interest must be trapped effectively and quantitatively during SFE, and the trapped analytes must be focused at the head of the analytical column as a narrow band to obtain good chromatographic performance in SFC.

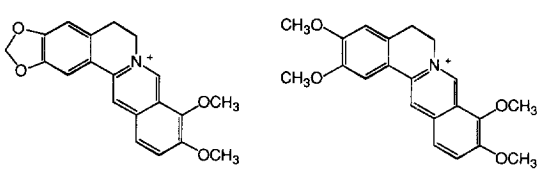

Fig. 1. Structures of berberine alkaloids in Phellodendri Cortex.

The goal of this study was selection of the best combination of entrainer, modifier and packing material for the column.

A method permitting rapid and efficient analysis of berberine and palmatine with IP-SFC on-line coupled with IP-SFE was established.

2. Experimental

2.1. Plant material

The commercial Phellodendri Cortex used in this study was purchased from Matsuura Yakugyo, Nagoya, Japan.

2.2. Chemicals and reagents

2.2.1. Solvents

Carbon dioxide was of a purity above 99.99% (Kanto Sanso, Tokyo, Japan). Methanol and acetonitrile were of HPLC grade (Wako, Tokyo, Japan). Chloroform, *n*-hexane and isopropanol were of spectrosol grade (Wako).

2.2.2. Solutes

Berberine and palmatine standards for quantitative determination were purchased from Wako.

Dioctyl sulfosuccinate sodium salt 98% was purchased from Aldrich Japan.

2.3. Apparatus

A supercritical fluid chromatograph, the Super 200 System 3 (Jasco, Tokyo, Japan) equipped with a 875-UV photometer (Jasco) was used.

2.4. On-line SFE–SFC conditions

2.4.1. SFE conditions

The extraction was performed for 10 min at a pressure of 20 MPa and a temperature of 60°C with supercritical carbon dioxide containing 10% (v/v) 100 m*M* DSS methanol solution at a flow-rate of 4 ml/min as liquid carbon dioxide. Extracts were directly passed through a silica gel column [GL-PACK LiChrosorb Si60-10 (5 μm), 150×4.6 mm I.D., GL Science, Tokyo, Japan], which was the SFC

column, and preconcentrated on the head of the column as a narrow band by the on-column trapping method.

2.4.2. SFC conditions

The extraction vessel was manually bypassed with a valve after SFE conditions were changed to those for SFC and the trapped analytes were consecutively analyzed. The only change in conditions was an increase in concentration of 100 m*M* DSS methanol solution in the mobile phase to 15% (v/v). Other conditions were the same as for SFE. Berberine and palmatine were UV monitored at 345 nm.

2.5. Assay procedure

About 2 mg of dry powder of Phellodendri Cortex, previously weighed accurately, were placed in the extraction cell [Cartridge Guard Column E, 35×4 mm I.D. (empty column), GL Science, Tokyo, Japan]. The sample was extracted and chromatographed under the same conditions as used for our on-line SFE–SFC system. The contents of berberine and palmatine were calculated from peak areas.

3. Results and discussion

The ion-pair technique has been used for solvent extraction and HPLC in the analysis of ionic compounds. Some reports have described the application of this technique to SFE and SFC [14,15]. We also tested various commonly used counter-ions used in HPLC. In SFC, the conditions had to be set above the limits of the solubility of the counter-ions in supercritical carbon dioxide (SF-CO_2). Most of them, such as sodium alkyl sulfonates used in analysis of alkaloids in reversed-phase HPLC, are difficult to dissolve in SF-CO_2 even if several decades of percentages of modifiers such as methanol are added to the fluid. DSS is a lipophilic surfactant and is highly soluble in nonpolar organic solvents. DSS was then tested as a counter-ion in our method. DSS had satisfactory solubility in organic solvents and mixed with SF-CO_2, and had selectivity for alkaloids. The IP-SFE method using DSS as the counter-ion was then used for the extraction of berberine and palmatine from Phellodendri Cortex.

If SFE extracts are trapped and focused on the head of the analytical column as a narrow band, it is possible to analyze them directly. DSS methanol solution as an entrainer was tested as a modifier in SFC. In order to achieve rapid stabilization of the system for the change from SFE to SFC, the same organic solvent was used and only its concentration was changed when changing from SFE to SFC. The concentration of the solvent must be high enough to increase the efficiency of extraction of analytes, but must not result in elution of the analytes from the column during extraction. It was also necessary to elute and separate the trapped analytes in SFC. In addition, the solvent concentration gradient between SFE and SFC had to be optimized.

3.1. SFE

3.1.1. Selection of ion-pair reagent

The ion-pair reagent most useful for ion-pair SFE was selected. Quarternary alkaloids like berberine and palmatine require neutralization of charged species by ion-pair formation with anionic counter-ions. Sodium dodecyl sulfate (SDS) and sodium 1-heptanesulfonate (SHS), which are widely used in ion-pair HPLC, and DSS, which has been used in reversed micellar chromatography, were considered for use as the counter-ion.

First, we studied the solubility of ion-pair reagents themselves in the supercritical carbon dioxide with polarity modified by methanol.

About 5 g of each ion-pair reagent were placed in a stainless-steel extraction vessel (30×50 mm I.D.). The extraction was performed for 5 min at a pressure of 20 MPa and at a temperature of 60°C with supercritical carbon dioxide containing 10, 15 or 20% (v/v) of methanol at a flow-rate of 4 ml/min as liquid carbon dioxide.

The extracts were collected and the contents of ion-pair reagents were weighed. Fig. 2 shows that, in the case of 10% methanol addition, DSS was extracted at more than 10 times the amount obtained with the other ion-pair reagents, while SDS and SHS were extracted to only a very small extent.

DSS is a surfactant which is readily soluble in nonpolar organic solvents such as *n*-hexane, and our findings showed that DSS had good solubility in SF-CO_2 modified methanol. DSS has double isooctyl

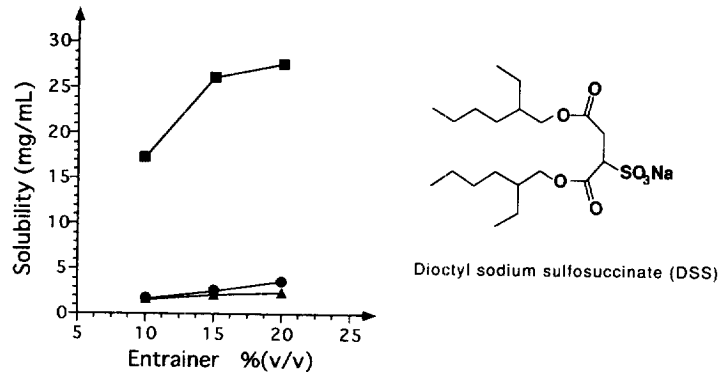

Fig. 2. Comparison of the solubility (mg/ml) of ion-pair reagents in the supercritical carbon dioxide modified with methanol. (■) DSS, (●) SDS, (▲) SHS. Conditions: 60°C, 20 MPa, flow-rate 4.0 ml/min as liquid carbon dioxide, added entrainer (10%, v/v) containing 100 m*M* DSS.

chains in its structure and is more lipophilic than SDS and SHS which have a single linear alkyl chain.

3.1.2. Polarity of entrainers

Methanol, isopropanol, chloroform and *n*-hexane were considered for use as the entrainer.

A 100 m*M* DSS solution of each entrainer was added to liquid carbon dioxide.

About 50 mg of dry powder of Phellodendri Cortex, previously weighed accurately, were placed in an extraction vessel [Cartridge Guard Column E, 35×4 mm I.D. (empty column), GL Science, Tokyo, Japan]. The extraction was performed for 10 min at a pressure of 20 MPa and a temperature of 60°C with supercritical carbon dioxide containing 10% (v/v) of 100 m*M* DSS solution of each entrainer at a flow-rate of 4 ml/min as liquid carbon dioxide.

The sample was extracted and each extract was collected. Yields of the alkaloids were compared among these entrainers. When 100 m*M* DSS solution of methanol was used as the entrainer, the yield of alkaloids was the highest and both berberine and palmatine were extracted at 3 times, 9 times or more than 50 times the amount obtained with 100 m*M* DSS solution of isopropanol, chloroform or *n*-hexane, respectively.

In the case of extraction performed without DSS, the yield of alkaloids with 10% (v/v) methanol alone as the entrainer was 1/4 the amount obtained with 100 m*M* DSS solution of methanol. With only 10% (v/v) isopropanol, chloroform or *n*-hexane as the entrainer the alkaloids were hardly extracted under our test conditions.

3.1.3. Physical parameters of supercritical fluid

3.1.3.1. Influence of pressure and temperature

As a general rule, if analytes are bonded to the matrix, greater solvating power is required for extraction, supercritical fluid density and/or extraction temperature is/are must be increased. Temperature and pressure were tested between 40 and 70°C and between 10 and 30 MPa, respectively.

An increase in temperature improved the efficiency of extraction, while an increase of pressure did not.

3.1.4. Effect of extraction time

The extraction rates for alkaloids with 10% (v/v) of 100 m*M* DSS methanol solution to liquid carbon as the extraction fluid were tested. The extraction time depended on the amount of sample and the volume of the extraction vessel. We have been developing an active and effective method of determination of components in crude drugs using on-line SFE–SFC. The purpose of the present study was to develop an extraction procedure for pretreatment in SFC analysis. For SFC analysis, only a few mg of sample were needed for this procedure. Extraction time was then determined with SFE with 2 mg of sample. The time required to obtain com-

plete extraction of the alkaloids from the crude drug was less than 10 min.

3.1.5. DSS concentration

Between 10 and 200 m*M* of DSS was added to methanol as the counter-ion. The resulting solutions were added as entrainers, and the extraction rates for berberine and palmatine were compared. The extraction rates were about the same from 10 to 200 m*M*, and an increase of DSS concentration did not affect the extraction rate in our test conditions.

Strong ionic compounds like quarternary alkaloids were only minimally extracted with SF-CO_2 alone. In addition, it is difficult to improve the extraction efficiency to achieve complete extraction of these compounds from the crude drug in a short extraction time, even if entrainers such as methanol are added. Neutralization of charged species by ion-pair formation is a way of reducing the polarity of ionic compounds and thus increasing their solubility in supercritical carbon dioxide when polar cosolvent addition is ineffective. Since DSS selectively neutralizes cationic compounds such as alkaloids, IP-SFE yields higher extraction efficiency for berberine and palmatine than SFE with SF-CO_2 modified with methanol.

3.2. On-line SFE–SEC

3.2.1. Comparison of retention behavior among combinations of the modifier and stationary phases

Combinations of DSS methanol solution as the modifier and NH_2 column or SiO_2 columns as the stationary phase were compared. The retention behaviors of berberine and palmatine were compared by adding the modifier to a concentration of 17.5%. Combination of DSS methanol solution with the SiO_2 column yielded satisfactory separation of analytes with a comparatively short retention time (Table 1), while the NH_2 column did not retain the alkaloids under the test conditions.

A more detailed study of the retention behavior of analytes with this combination was carried out by changing the concentration of the modifier. With SiO_2 columns, the k of berberine was above 20 with addition of the modifier to less than 10% concentration. Berberine was retained to a high degree on the head of the SiO_2 column. In on-line SFE–SFC, if the extraction time was short, berberine and palmatine were focused at the head of the column and they were eluted and completely separated within about 10 min when increasing the concentration of DSS methanol solution above 15%.

Table 1
Comparison of retention behavior on berberine and palmatine in various stationary phases

Stationary	t_0	Capacity factor (k)	
		Berberine	Palmatine
Capcell Pak NH_2 SG80	0.80	0.35	0.31
Capcell Pak CN SG120	0.69	0.66	0.65
Capcell Pak SILICA SG120	0.91	2.95	3.58
GL-Pack LiChrosorb Si60	0.82	6.88	8.20

1,3,5-Tri-*tert.*-butylbenzene was used to obtain t_0 for calculating k with UV detection at 254 nm.

3.2.2. Determination of berberine and palmatine in Phellodendri Cortex

Fig. 3 illustrates the results of separation and determination results of berberine and palmatine for two samples of Phellodendri Cortex on the market.

The samples were produced in Japan, and the

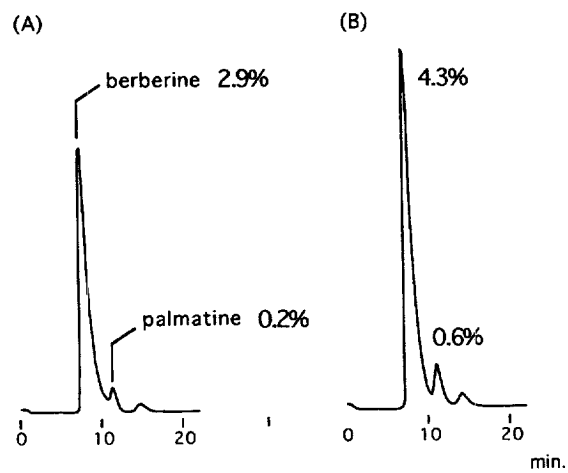

Fig. 3. Contents of berberine and palmatine in Phellodendri Cortex on the market. (A) Niigata, Japan, (B) Fukui, Japan. SFE conditions: 60°C; 20 MPa; flow-rate, 4.0 ml/min; added methanol (10%, v/v), containing 100 m*M* DSS; extraction time, 10 min; sample, 2 mg. SFC conditions: 60°C; 20 MPa; flow-rate, 4.0 ml/min; added methanol (15%, v/v), containing 100 m*M* DSS; column GL-Pack LiChrosorb Si60 (150×4.6 mm I.D., 10 μm); detection, 345 nm.

content of berberine was about ten times the amount of that of palmatine. The results were also almost the same as those obtained with the HPLC method with the solvent extraction reported in the literature [10].

3.2.3. Calibration curve

Calibration curves for both berberine and palmatine were obtained from 0.71–50.49 μg and 5.04–21.84 μg, respectively. The respective regression equations were as follows: $y=11\ 505\ 000x+217\ 000$ ($r=0.999$) and $y=5\ 802\ 000x+224\ 000$ ($r=0.999$), where y is peak area and x is concentration (μg).

4. Conclusion

Using the system described, with IP-SFC on-line coupled to IP-SFE, it was possible to determine the concentration of berberine and palmatine in Phellodendri Cortex using DSS as the counter-ion with a SiO_2 column for trapping and analysis. The SiO_2 column exhibited strong retention of berberine and palmatine, and these analytes were focused at the column head during SFE, even if 10% (v/v to liquid carbon dioxide) of 100 m*M* DSS methanol solution was added as the entrainer. After extraction, the analytes were desorped quickly, and SFC was successfully performed by increasing the modifier concentration to 15%.

In this study, the analytes were focused at the column head if the addition of modifier gave k values higher than 20.

Berberine and palmatine in Phellodendri Cortex were determined from only a few mg of crude drug powder. Ten minutes were needed for the SFE extraction, and 10 min for the SFC analysis. Thus, within ca. 20 min, rapid analysis was completed, from extraction as a pretreatment through analysis, including the system change from SFE to SFC.

DSS could be used as a counter-ion in our ion-pair method and with it the efficiency of extraction and selectivity for alkaloids were greatly improved. It was possible to apply DSS to achieve ion-pair formation with cationic compounds which could not be extracted with SF-CO_2 or modified SF-CO_2 with cosolvents alone. The limit of the ionic analyte was greatly spread by this on-line IP-SFE–IP-SFC system. It appears that our system will be extremely useful for the analysis of alkaloids in herbal medicines and will in particular permit rapid analysis including extraction of the analytes as a pretreatment.

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