ELSEVIER

Journal of Chromatography A, 810 (1998) 252–255

JOURNAL OF CHROMATOGRAPHY A

Short communication

Determination of atractylon in Atractylodes rhizome using supercritical fluid chromatography on-line coupled with supercritical fluid extraction by the direct induction method

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 Company, 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 22 September 1997; received in revised form 4 February 1998; accepted 5 March 1998

Abstract

Atractylon was extracted from Atractylodes rhizome within 15 s with supercritical carbon dioxide. The extraction was so rapid that SFE extracts could be transported directly into an SFC column and analyzed. Atractylon was eluted and separated in ca. 5 min. Analyte recovery was 30% higher than when using solvent extraction. Determination of atractylon in Atractylodes rhizome was achieved with only a few mg of crude drug sample. Atractylon is unstable due to oxidation and is easily transformed to atractylenolides. Loss of analytes during the procedures was also minimal with this on-line SFE–SFC system.

Keywords: Atractylodes rhizome; Atractylon

1. Introduction

We have published a rapid and efficient method for characterization of several herbal medicines using SFE and SFC [1–5]. In these reports, on-line SFE–SFC systems with trapping column or on-column trapping methods were used as the coupled system. If the period of time for SFE extraction of analytes from crude drug samples is very short, it is possible to transport SFE extracts into an SFC column directly without intermediate trapping and concentration of the extracts. We achieved SFE of atractylon in Atractylodes rhizome within 15 s and established a method for determination of atractylon using the on-line SFE–SFC system. Atractylodes rhizome is a useful drug prescribed in many Japanese and Chinese herbal medicines. Atractylon [6] is a characteristic component of Atractylodes rhizome (Fig. 1) which is readily transformed to atractylenolides by oxidation and is also degraded by UV light irradiation. Analytical HPLC or GC methods [7] require manual extraction of the analyte from the crude drug prior to chromatographic analysis. In this report, the application of our on-line SFE–SFC system to the quantitative determination of atractylon in commercially available Atractylodes rhizome is described. This

*Corresponding author.

0021-9673/98/$19.00
PII: S0021-9673(98)00225-8

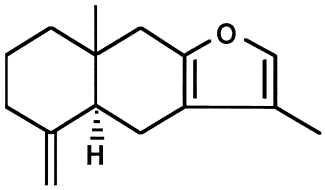

Fig. 1. Structure of atractylon.

on-line SFE–SFC system features inert and mild conditions during SFE and SFC procedures. This characteristic is very important for the analysis of analytes which are thermally labile and/or unstable to oxidation.

2. Experimental

2.1. Plant material

The commercial Atractylodes rhizome used in this study was purchased from Alps Pharmaceutical (Gifu, Japan).

2.2. Chemicals and reagents

2.2.1. Solvents

Carbon dioxide was of purity above 99.99% (Kanto Sanso, Tokyo, Japan) and methanol was of HPLC grade (Wako, Tokyo, Japan).

2.2.2. Solute

Atractylon standard for quantitative determination was purchased from Wako.

2.2.3. Apparatus

A supercritical fluid chromatograph SUPER 200 system 3 (JASCO, Tokyo, Japan) equipped with an 875-UV photometer (JASCO) was used.

2.2.4. Column

An SiO_2 column [LiChrosorb Si60-10 (250 mm×4.6 mm I.D., 10 μm, Merck) and TSK gel Silica-60 (250 mm×4.6 mm I.D., 5 μm, Tosoh)], an ODS column [Mightysil RP-18 (250 mm×4.6 mm I.D., 5 μm, Kanto Chemical) and Inertsil ODS-2 (250 mm×4.6 mm I.D., 5 μm, GL Science)], a C_8 column [LiChrospher 100 RP 8(e) (250mm×4.6 mm I.D., 10 μm, Merck)] and a Diol column [LiChrospher Diol-5 (250 mm×4.6 mm I.D., 5 μm, Merck)] were used.

2.3. SFE and SFC conditions

The extraction and analysis were performed at the same time and under the same conditions. A pressure of 10 MPa was used with a temperature of 40°C with supercritical carbon dioxide at a flow-rate of 2 ml/min as liquid carbon dioxide. Atractylon was UV-monitored at 220 nm. SFE was performed for 15 s and the extraction cell was immediately bypassed after SFE. A SiO_2 column was used as the SFC column.

2.4. Assay procedure

About 2 mg of dry powder of Atractylodes rhizome, previously weighed accurately, was placed in the extraction cell (Cartridge Guard Column E, empty column, 10 mm×4 mm I.D., GL Science, Tokyo, Japan). The sample was extracted and chromatographed under the conditions described above. Atractylon content was calculated from the peak areas. Atractylon standard was dissolved in *n*-hexane and used as a standard solution.

3. Results and discussion

First, the SFE–SFC system was tested with a trapping column. In this method, the extract was trapped on the column bed by reduction of fluid solubility following sudden drop in pressure to atmospheric pressure in the trap column. Extraction of the analytes was easily and completely performed in a short period of time with only supercritical carbon dioxide. However, there was some loss of atractylon in the trapping procedure, and the extraction yield compared with solvent extraction [7] was ca. 90%.

3.1. Selection of SFC column

The SiO_2 column, ODS column, C_8 column and the Diol column were compared for the stationary phase. Since there was some loss of components in

column trapping and collection of fractions, tests of recovery and separation of the crude drug sample were directly performed with directly transported on-line SFE–SFC.

About 10 mg of dry powder of the crude drug were placed in the extraction cell. SFE was by-passed and the SFC column was conditioned. After conditioning, the SFE switching valve was switched to on-line SFE–SFC. SFE and SFC were simultaneously begun and the extraction cell was bypassed again after 5 min. A pressure of 10 MPa was used with a temperature of 40°C with supercritical carbon dioxide at a flow-rate of 2 ml/min. Atractylon was UV-monitored at 220 nm.

Few components were eluted in chromatograms obtained, and it was presumed that the combined selectivity of SFE and SFC for atractylon in the crude drug was relatively high. As the SiO_2 column (TSK gel Silica-60) yielded the best separation of analytes and peak shapes for the SFC profile. This column was selected for further work.

3.2. Pressure and temperature

Using the SiO_2 column, a more detailed study of retention behavior and separation of atractylon in crude drug sample was performed with changes in pressure and temperature. About 10 mg of dry crude drug powder was placed in the extraction cell. The extraction cell was by-passed 5 min after initiation of on-line SFE–SFC analysis. Test conditions included pressures from 8 MPa to 15 MPa and temperatures from 40°C to 60°C, with supercritical carbon dioxide at a flow-rate of 2 ml/min.

With increases in temperature, retention of atractylon increased and peak shape became broad in the chromatogram. Extraction yields decreased with increases in temperature.

With increases in pressure, the retention time of atractylon became shorter and markable peak tailing was observed, and it was difficult to separate it completely from other components in the crude drug sample. A pressure of 10 MPa and temperature of 40°C gave the best peak shape and complete separation of atractylon from other components using our test conditions.

3.3. Extraction time

Diffusion of atractylon in the SFC column during extraction appeared to be improved by reduction of the extraction time for complete extraction of atractylon from the crude drug. Extraction time depends on sample amount and extraction cell volume. Sample amounts of 2–10 mg were tested. A small extraction cell (Cartridge Guard Column E, empty column, 10 mm×4 mm I.D., GL Science, Tokyo, Japan) was used, and the free space of the cell was packed with sililated glass wool. A 2 mg sample exhibited the highest extraction yields at extraction times of more than 5 s, and 15 s was sufficient for extraction of the analyte from 2 mg of crude drug sample in SFE (Fig. 2). The extraction yield at 15 s was ca. 30% higher than that obtained by solvent extraction and GC analysis. The peak of atractylon on SFC was isolated and reanalyzed by HPLC, and was a single peak without other components. In a comparison of extraction profiles (Fig. 3), with 2 mg, complete extraction of total extracts containing atractylon was achieved within 15 s and the extraction yield of atractylon also reached equilibrium at 15 s. With 5 mg and 10 mg, almost all extracts in the crude drug sample were extracted rapidly, but the residue, which was small in amount, was slowly extracted. Although the profiles did not show that of atractylon itself, with 5 mg and 10 mg, it was presumed that more than 15 s was required for complete extraction of atractylon, and the atractylon extracted after more than 15 s was detected not as a

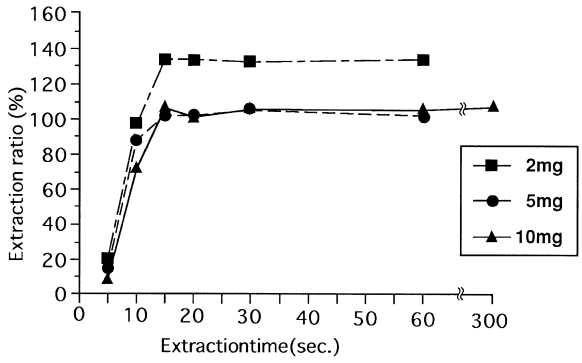

Fig. 2. Comparison of extraction yields with various sample amounts and extraction time.

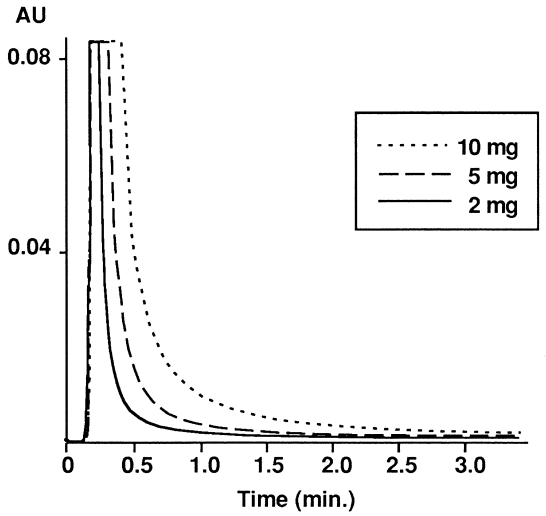

Fig. 3. Comparison of extraction profiles with various sample amounts. Profiles were UV-monitored at 220 nm.

peak but rather as a peak tailing or increased baseline in this method.

3.4. Determination of atractylon in Atractylodes rhizome

Two Chinese and three Korean samples were tested. Fig. 4 shows an SFC profile and contents of atractylon in a commercially available Atractylodes rhizome produced in China (Ji Lin). All samples showed an almost identical chromatographic pattern and atractylon could be determined without any disturbance. Atractylon contents of three Korean samples were 0.4%, 1.2% and 1.1%, respectively. A calibration curve for the atractylon standard was

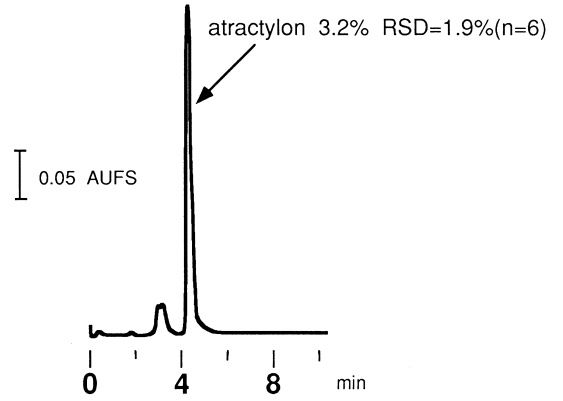

Fig. 4. SFC profile of Atractylodes rhizome (produced in Ji Lin, China).

obtained for 6.3–18.9 μg. The regression equation was $y=137\,900x+40\,700$ (0.999), where y is the peak area and x is the concentration (μg).

References

[1] K. Suto, K. Sagara, T. Mizutani, Nat. Med. 45 (1991) 29.
[2] K. Suto, M. Masuda, T. Maruta, T. Mizutani, Nat. Med. 46 (1992) 9.
[3] K. Suto, Y. Ito, K. Sagara, H. Itokawa, J. Chromatogr. A 786 (1997) 366.
[4] K. Suto, S. Kakinuma, Y. Ito, K. Sagara, H. Iwasaki, H. Itokawa, J. Chromatogr. A 786 (1997) 371.
[5] K. Suto, S. Kakinuma, Y. Ito, K. Sagara, H. Iwasaki, H. Itokawa, Chem. Pharm. Bull. 45 (1997) 1708.
[6] H. Hikino, Y. Hikino, I. Yoshioka, Chem. Pharm. Bull. 10 (1962) 641.
[7] N. Hiraoka, K. Matoba, N. Ogawa, Shoyakugaku Zasshi 47 (1993) 65.