Use of Dioctylsulphosuccinate Sodium Salt in Supercritical Fluid Chromatography

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Ion-pair reagents and reversed micelles in supercritical carbon dioxide ($SF\text{-}CO_2$) were tested as polar mobile phases for supercritical fluid chromatography (SFC). Dioctylsulphosuccinate sodium salt (DSS) was used as the counter-ion in ion-pair supercritical fluid chromatography (IP-SFC) with a packed silica-gel column. DSS/pentane reversed micelles were used in reversed micelle supercritical fluid chromatography (RM-SFC) with a packed fluoroalkyl silica-gel column. IP-SFC and RM-SFC using DSS was demonstrated for analytical separation of ephedrine alkaloids.

Key words dioctylsulphosuccinate sodium salt; ion-pair supercritical fluid chromatography; reversed micelle supercritical fluid chromatography; ephedrine alkaloid

Supercritical fluid chromatography (SFC) can be used to separate thermally labile and high-molecular-weight samples unsuitable for GC analysis. Compared to HPLC, it offers high separation efficiencies, shorter analysis times, and a wider range of possibilities of detection.[1] We have reported a rapid and efficient method for characterization of herbal medicine using supercritical fluid extraction (SFE) and SFC.[2,3] In this report, we describe the use of ion-pair supercritical fluid chromatography (IP-SFC)[4,5] and reversed micelle supercritical fluid chromatography (RM-SFC).[6]

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 addition of polar cosolvent is ineffective.

Dioctylsulphosuccinate sodium salt (DSS) is a surfactant and is highly soluble in nonpolar organic solvents. We therefore tested its use as a counter-ion in IP-SFC. DSS had satisfactory solubility in supercritical carbon dioxide modified with methanol and satisfactory selectivity for ephedrine alkaloids.

We used IP-SFC with DSS as the counter-ion for analysis of ephedrine alkaloids (Fig. 1). Commercial Ephedrae Herba was tested for analytical separation of the analytes, and results confirmed that our IP-SFC method could be used for the analysis of ephedrine alkaloids in Ephedrae Herba.

As another means of modifying polarity of the mobile phase, RM-SFC was tested using DSS reversed micelles. Reversed micelles were produced by addition of a small amount of water to DSS pentane solution. Analytical separation of ephedrine alkaloids was tested, and results showed that RM-SFC could be used for the analysis of ephedrine alkaloids.

Experimental

Plant Material The commercial Ephedrae Herba used in this study was purchased from Matsuura Yakugyo, Nagoya, Japan.

Chemicals and Reagents Solvents: Carbon dioxide was of high purity above 99.99% (Kanto Sanso Ind., Tokyo, Japan). Methanol was of HPLC grade (Wako Pure Chemical Ind., Tokyo). Pentane and diethyl ether were of reagent grade (Wako).

Solutes: Hydrochlorides of ephedrine, methylephedrine and norephedrine were purchased from Fuji Pharmaceuticals, Tokyo. Pseudoephedrine hydrochloride was purchased from Alps Pharmaceutical Ind., Gifu, Japan. Dioctylsulfosuccinate sodium salt (98% purity) was purchased from Aldrich Japan, Tokyo.

Column: A Finepak SIL-5P (5 μm, 250 × 4.6 mm I.D., JASCO, Tokyo, Japan) was used for IP-SFC, while a Fluofix 120E (5 μm, 50 × 4.6 mm I.D., NEOS, Kobe, Japan) was used for RM-SFC.

Apparatus A supercritical fluid chromatograph, the SUPER 200 System 3 (JASCO) equipped with an 875-UV photometer (JASCO), was used.

Procedure for Obtaining Crude Drug Test Solution Approximately 0.5 g of dry powder of Ephedrae Herba was weighed in a glass-stoppered centrifuge tube. Fifteen ml of 0.1 N NaOH solution and 15 ml of diethyl ether were mixed and shaken for 30 min. The diethyl ether layer was separated by centrifugation. This procedure was repeated twice for the residual NaOH solution layer. The diethyl ether layer was combined, and diethyl ether was added to make exactly 50 ml. This solution was used as the test solution of the crude drug.

Standard Solution Approximately 0.025 g portions of ephedrine hydrochloride, pseudoephedrine hydrochloride, methylephedrine hydrochloride and norephedrine hydrochloride (dried at 105 °C for 3 h) were weighed and dissolved in methanol to make exactly 10 ml. This was used as the standard solution.

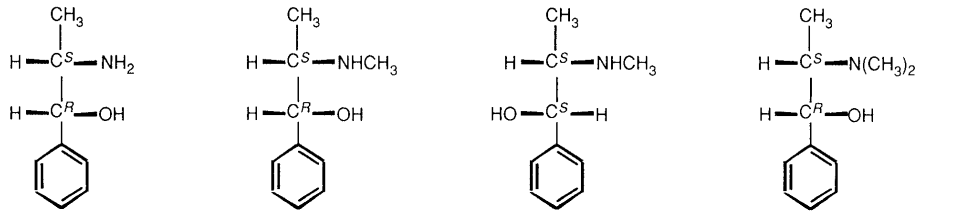

Fig. 1. Structures of Ephedrine Alkaloids

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Results and Discussion

IP-SFC The ion-pair technique has been used for solvent extraction and HPLC in the analysis of ionic compounds.[7-9] Some reports have described the application of this technique to SFE and SFC.[4,5,10] We also tested various counter-ions commonly used in HPLC or LC-MS. First, the solubility of the counter-ions in SF-CO_2 modified with some modifiers was tested. Sodium alkyl sulfonates such as sodium dodecylsulfonate have been used in analysis of alkaloids in reversed phase ion-pair HPLC.[11] Although most of these sulphonates have a single linear alkyl chain, they were difficult to dissolve in SF-CO_2 even if a percentage of tens of a modifier such as methanol was added to the fluid.

Fluoroalkylsulfonates used in LC-MS were soluble in SF-CO_2 modified with methanol, but they did not have satisfactory selectivity for ephedrine alkaloids under the SFC conditions we used.

DSS, which has double branched alkyl chains, is a lipophilic surfactant and is highly soluble in nonpolar organic solvents. It was therefore tested as a counter-ion using our method. DSS had satisfactory solubility in a small amount of organic solvent. Methanol solution of DSS was mixed with SF-CO_2 as a modifier with a selectivity for ephedrine alkaloids in SFC.

Application of this ion-pair technique to SFC analysis of ephedrine alkaloids was tested.

IP-SFC Analysis DSS Concentration: DSS (between 5 and 100 mM) was added to methanol as the counter-ion. The resulting solutions were added as the modifier, and the retention behaviors of ephedrine alkaloids were compared. (Fig. 2)

The retention behaviors of ephedrine alkaloids were studied by changing the concentration of DSS. The k' of norephedrine was more than about 20 with the addition of less than 5 mM DSS. Ephedrine alkaloids were retained to a high degree on the silica-gel column and their peaks were too broad to detect. Their peak shapes were improved by increasing DSS concentration, and alternate analytical separation in a short period was successful (Fig. 3). Chromatographic behavior based on the ion-pair formation was observed at DSS concentrations above 5 mM in the modifier. Figure 4 illustrates the result of separation of ephedrine alkaloids for a sample of commercially available Ephedrae Herba.

RM-SFC: Reversed micelle formation in supercritical solvents with low critical temperatures is an additional means of modifying the mobile phase in SFC. Pentane reversed micelle mobile phase was prepared by dissolving the appropriate weight of DSS (0.01 M) in pentane and adding water to obtain a surfactant-to-water ratio W (=[H_2O]/[DSS]) of 5.0. Analytical separation of ephed-

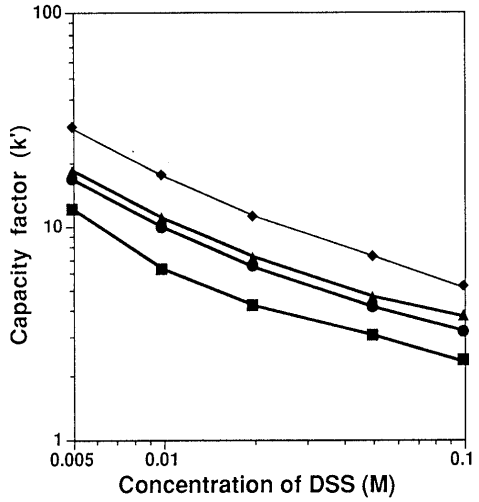

Fig. 2. Effect of DSS Concentration

□, norephedrine; ●, ephedrine; ▲, pseudoephedrine; ◆, methylephedrine. IP-SFC conditions: mobile phase, CO_2 (3.5 ml/min); modifier, DSS/MeOH (0.5 ml/min); column, TSKgel Silica-60 (250 × 4.6 mm I.D.); pressure, 20 MPa; column temp., 60 °C; detector, UV 254 nm.

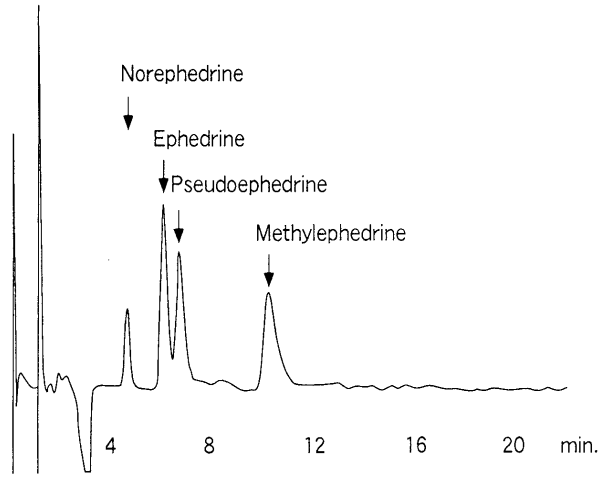

Fig. 3. IP-SFC Profile of Ephedrine Alkaloids

IP-SFC conditions: mobile phase, CO_2 (3.5 ml/min); modifier, 0.1 M DSS/MeOH (0.5 ml/min); column, Finepak SIL-5P (50 × 4.6 mm I.D., 5 μm); pressure, 20 MPa; column temp., 60 °C; detector, UV 254 nm.

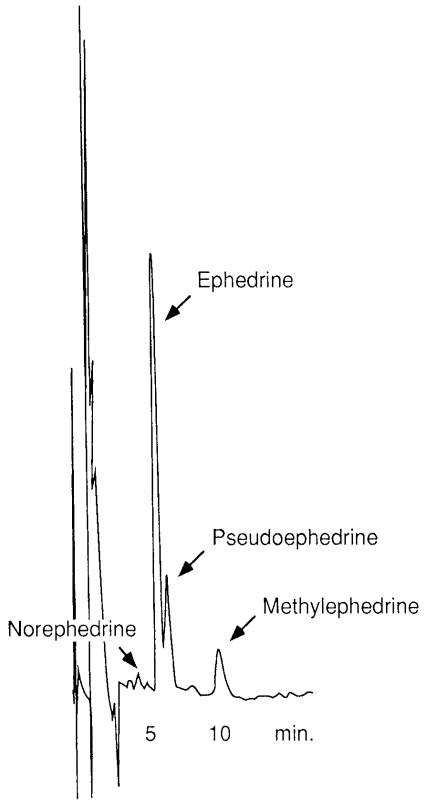

Fig. 4. IP-SFC Profile of Ephedrine Alkaloids in Ephedrae Herba

SFC conditions were the same as those in Fig. 3.

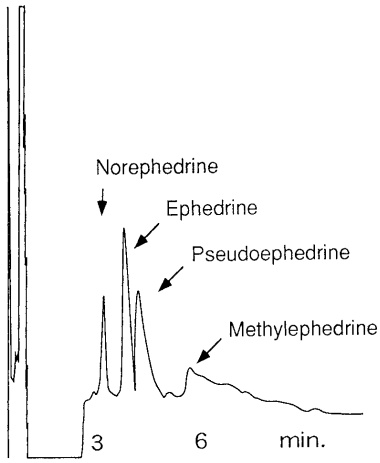

Fig. 5. RM-SFC of Ephedrine Alkaloids

RM-SFC conditions: mobile phase, CO_2 (2.8 ml/min); modifier, pentane (0.03 M DSS, 0.15 M H_2O) (1.2 ml/min); column, Fluofix 120E (50 × 4.6 mm I.D., 5 μm); pressure, 20 MPa; column temp., 60 °C; detector, UV 254 nm.

rine alkaloids was then tested.

RM-SFC Analysis Figure 5 illustrates the result of separation of ephedrine alkaloids. We confirmed that they were separated and eluted in RM-SFC.

Large changes in retention were observed as previously reported at DSS concentrations below the critical micelle concentration.[12] In addition, the p*K*a (strength of amine) of each alkaloid affected partitioning into the micelle. It is anticipated that selectivity may be adjusted by using pH, ionic strength, or supercritical fluid pressure to control solute-micelle partition, as true for control of selectivity in extraction processes.[13-16]

Conclusion

DSS could be used as a counter-ion in our IP-SFC, and yielded ion-pair formation with ephedrine alkaloids in SFC. They were more efficiently separated and eluted in IP-SFC than SFC with modified SF-CO_2 using cosolvents alone. The limit of the ionic analyte in normal phase SFC analysis was greatly improved with this IP-SFC method. These findings suggest that our system will be extremely useful for the analysis of alkaloids in herbal medicines.

As another means of modifying mobile phase polarity, RM-SFC using DSS reversed micelles also achieved analytical separation of ephedrine alkaloids. Thus, it appears that this system will also be beneficial for SFC analysis of polar compounds in herbal medicines.

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