Analysis of Medicine in Serum by Online SFE-SFC

Introduction

In recent years, DBS (Dried Blood Spots) method is attracting attention as one of the method to measure blood concentration of medicines and the like. DBS method has advantages such as a small amount (15 to 20 µL) of required sample volume, no need for centrifugation of plasma, and transport and storage at the room temperature can be available. Usually in this method, sample is dropped onto a dedicated DBS card, dried and hollowed out, and solvent extraction is performed.

On the other hand, supercritical fluid extraction (SFE) is an extraction method using supercritical carbon dioxide as a main solvent, high efficient extraction can be possible by characteristics of low viscosity, high diffusivity and high solubility of supercritical fluid. In recent years, with the deregulation of High Pressure Gas Safety Act, interest in SFE and supercritical fluid chromatography (SFC) has increased, and expansion of its application range is desired.

In this note, the result of analysis of medicine in serum by DBS (Dried Blood Spots) method using Online SFE-SFC system which can perform pretreatment by SFE and separation analysis by SFC was reported.

Keyword : Flurbiprofen, ketoprofen, warfarin, propranolol, DBS, SFCpak SIL DIOL-5, supercritical fluid extraction, supercritical fluid chromatography, Online SFE-SFC

Experimental condition

[SFE Conditions]

Extraction vessel:	EV-DBS (Order Made)
Extraction solvent:	CO_2/0.3% Diethylamine in Methanol (3.2/0.3)
Oven temp.:	35˚C
Wavelength:	210 nm
Pressure:	20 MPa

[SFC Conditions]

Column:	SFCpak SIL DIOL-5 (4.6 mmI.D. x 250 mmL , 5 µm)
Eluent:	CO_2/0.3% Diethylamine in Methanol (3.2/0.3)
Flow rate:	3.5 mL/min
Column temp.:	35˚C
Wavelength:	220-400 nm Max Absorbance 245 nm: Flurbiprofen 250 nm: Ketoprofen 275 nm: Warfarin 290 nm: Propranolol
Pressure:	20 MPa
Standard solution:	Flurbiprofen, Ketoprofen, Warfarin, Propranolol

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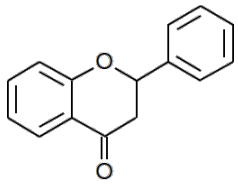
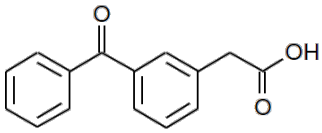
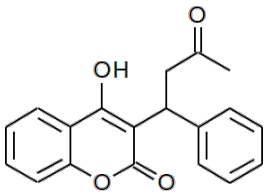
Structure



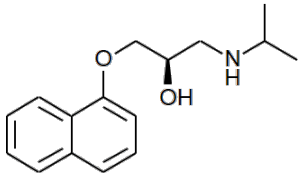
Flurbiprofen
(IS: Internal Standard)



Ketoprofen



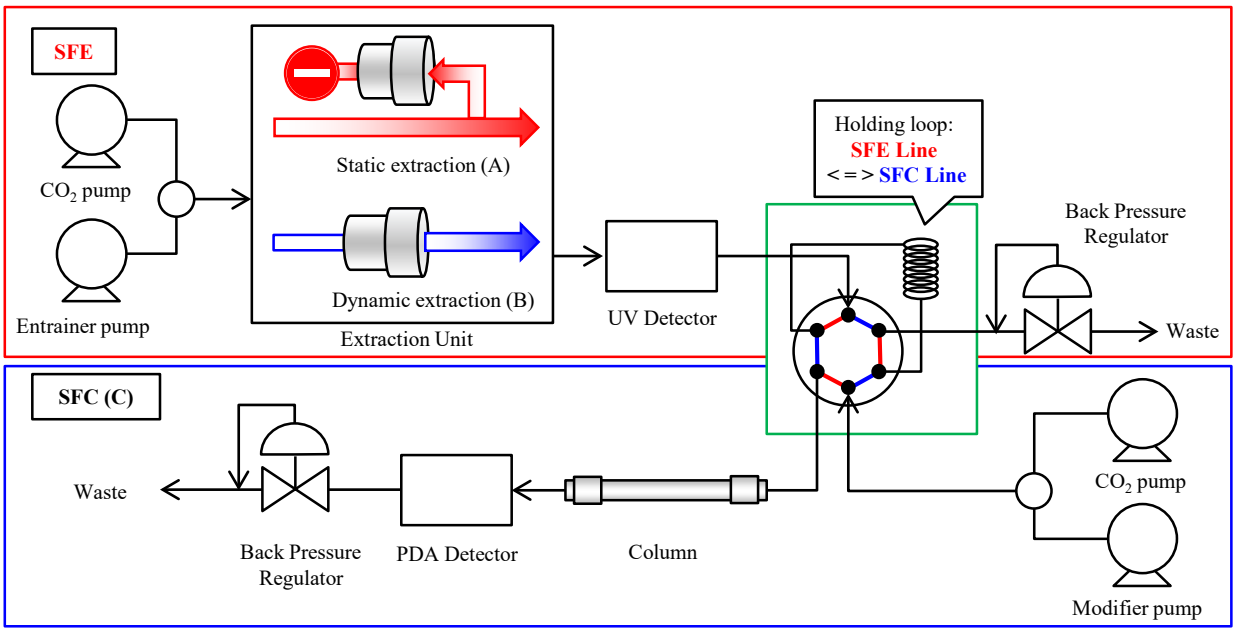
Warfarin



Propranolol

Schematic diagram

Figure 1-1 shows the flow diagram. SFE was performed combining static extraction (2 minutes) and dynamic extraction (Figure 1-2). The introduction of extract to SFC adopts heart cut method. At the timing when the target component extracted by SFE passes through the holding loop, the valve is switched and the component is introduced into the SFC flow path (Figure 1-3). By monitoring the extraction pattern by SFE using a UV detector and switching the valve at optimal timing, it is possible to introduce extracts to the SFC at a higher recovery rate.



1) Load the sample into the extraction container and connect it to the SFE flow path
2) Perform static extraction on the SFE system (A)
3) Perform dynamic extraction on the SFE system (B)
4) Check the extraction pattern and switch the valve at the timing when the extract passes through the holding loop
5) Introduce the extract filled in the loop into the column (C)

Fig. 1-1 System outline of online SFE-SFC (flow diagram)

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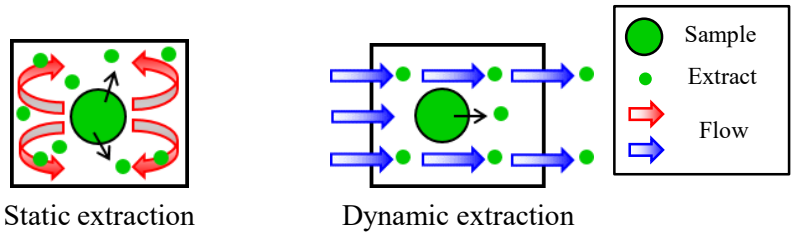

Fig. 1-2 System outline of online SFE-SFC (image of static and dynamic extraction)

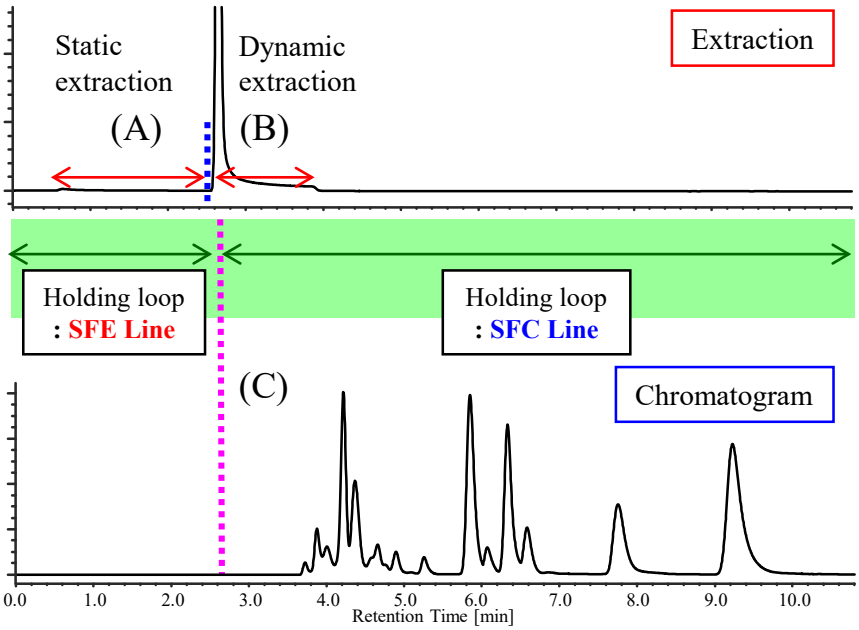

Fig. 1-3 System outline of online SFE-SFC (image of extraction-chromatograph)

5 µL of a standard solution (Flurbiprofen (internal standard (IS)), Ketoprofen, Warfarin, Propranolol, methanol solution) was dropped on a DBS card hollowed out to 3 mm in diameter using a dedicated punch for DBS, and the card was dried. This card was loaded in an extraction container dedicated to DBS, connected to SFE flow path, extraction and analysis were carried out. The procedure is shown below.

1) Hollow out the DBS card to 3 mm in diameter using dedicated punch (MICRO-PUNCH).
2) Overlap the two hollowed out disc-shaped DBS cards and drop 5 µL of the sample to it.
   (10, 25, 50, 100 mg / L STD)
3) Leave it to dry. (Sample dissolution solvent: about 20 minutes in case of methanol, about 60 minutes in case of aqueous system)
4) Set the dried disc-shaped DBS card in a dedicated extraction container (EV-DBS).

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Results

Figure 2 show the chromatograms of the standard solution. The top is the extraction pattern, the bottom is the chromatogram of the extracted standard solution.

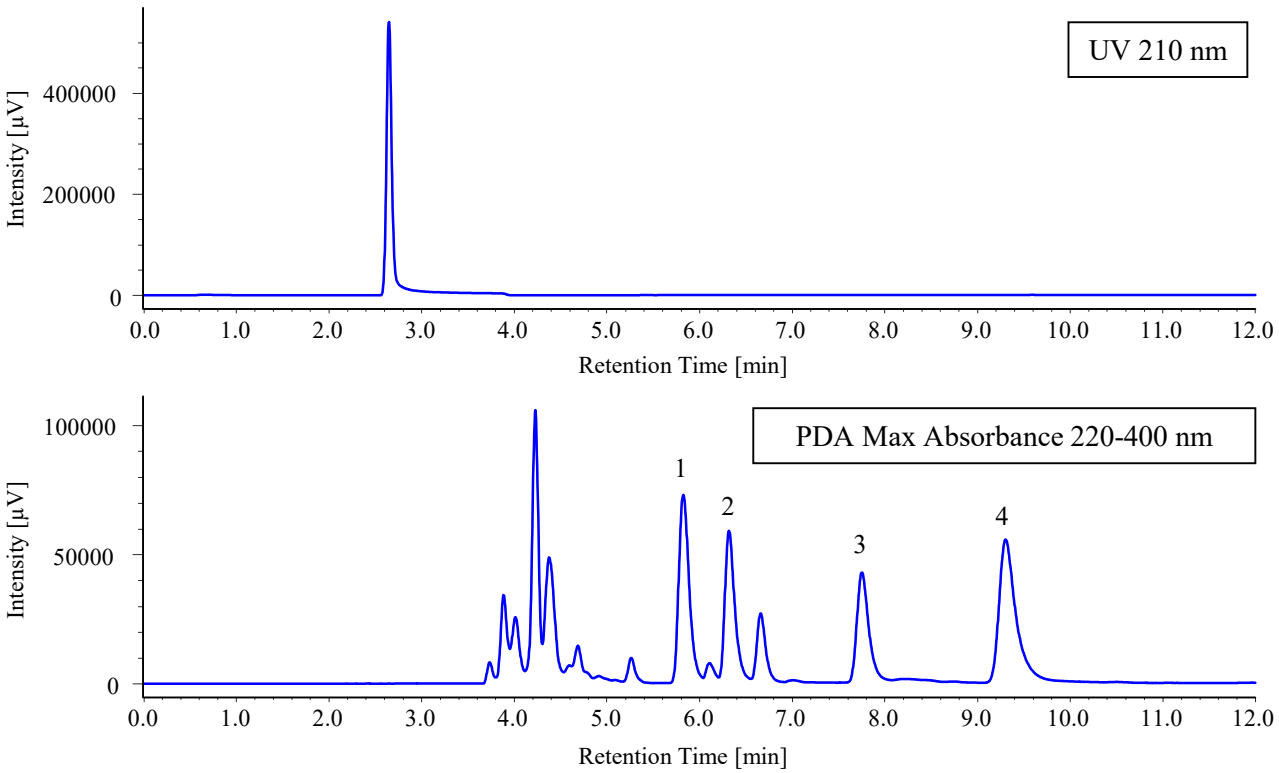

Fig.2 Chromatograms of the standard solution
1: Flurbiprofen, 2: Ketoprofen, 3: Warfarin, 4: Propranolol

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A standard solution was added to control serum (FUJIFILM Wako Pure Chemical Corporation) and an addition recovery experiment was performed. Figure 3 show the Max Absorbance chromatograms of each sample in the addition recovery experiment. Since propranolol overlapped with the serum unknown component at the detection wavelength of 290 nm, the recovery rate was calculated at 230 nm with little influence of serum unknown components. As a result, a recovery rate of more than 90% was obtained for all components (Table 1).

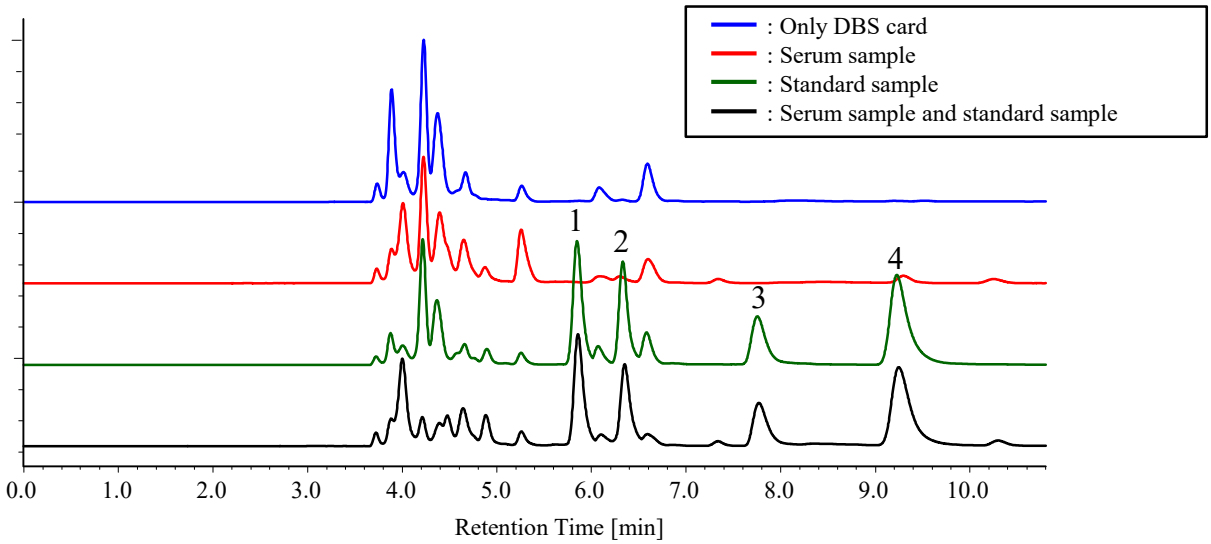

Fig. 3 Max Absorbance chromatogram of each sample (220 to 400 nm)
1: Flurbiprofen, 2: Ketoprofen, 3: Warfarin, 4: Propranolol

Table 1 Recovery rate in addition recovery experiment

Sample No.		(B)*1	(C)*1	Recovery rate*2 (%)
Flurbiprofen	IS 245 nm	1	1	1
Ketoprofen	250 nm	0.7765	0.7385	95.1
Warfarin	275 nm	0.373	0.3487	93.5
Propranolol	290 nm	0.2333	0.2625	112.5
Propranolol	230 nm	0.9771	0.9049	92.6

* 1: Sample area/IS area
* 2: (3)/(2) x 100

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