# High Sensitivity Fluorescence Detector for Supercritical Fluid Chromatography

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## Introduction

Fluorescence detection has been widely used as one of a practical detection method in HPLC analysis. It enables the selective detection of fluorescent substances, with sensitivity that can be up to 1000 times greater than with UV detection.

Fluorescence detection can also be applied to non-fluorescent substances with the use of derivatization, with many derivatization agents becoming commercially available in recent years. Therefore, this technique provides the advantages of highly sensitivity and selectivity to many different classes of compound, and broadens the range of applications in many industries. However, fluorescence detection has not been used in SFC analysis due to the difficulty of developing a suitable high pressure flow cell.

We developed a fluorescence detector with a flow cell specifically constructed for operations at the high pressures required for SFC. This fluorescence detector is included in our newer SFC system (SFC-4000 series). In this presentation, we will introduce an application for the analysis of several compounds using SFC separation with fluorescence detection.

## Experimental

### Apparatus

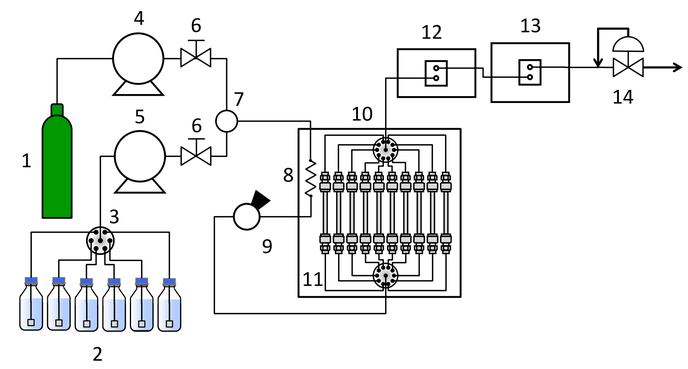
Figure 1 shows the JASCO SFC system equipped with UV/Visible (UV) and fluorescence (FL) detectors used in this experiment. Figure 2 also shows the schematic diagram of this system. This system enables method scouting analysis to be performed on several mobile phases and columns.

Figure 3 shows the structures and differences between a conventional and novel fluorescence detector flow cell. We developed a unique quartz flow cell design and structure for use with high pressure (up to 20 MPa). The outer surfaces of this flow cell are coated with aluminum, and provides high collection efficiency of fluorescence by functioning as a reflector.

$CO_2$ delivery pump : **PU-4380**
Modifier solvent delivery pump : **PU-4180** (with SV*1, LV*2 and MX*3 unit)
Autosampler : **AS-4350**
Column oven : **CO-4065** (with 2 switching valve unit*4)
Detector 1 : **UV-4070** (with Analytical High Pressure cell)
Detector 2 : **FP-4020** (with Analytical High Pressure cell)
Back pressure regulator : **BP-4340**
Chromatography data system : **ChromNAV Ver.2**

*1 Stop valve unit *2 Solvent switching unit
*3 Mixing unit *4 10 position-11port valve unit

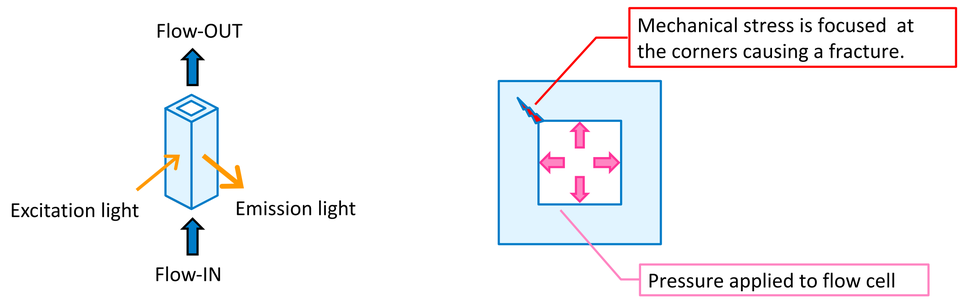
Fig.1 The JASCO SFC system ( EXTREMA )




1 : $CO_2$ cylinder
2 : Modifier solvents
3 : Solvent switching valve
4 : $CO_2$ delivery pump
5 : Modifier solvents delivery pump
6 : Stop valve
7 : Mixing unit
8 : Preheat coil
9 : Autosampler
10 : Column oven (with 2 switching valve units)
11 : Columns
12 : UV/Vis detector
13 : Fluorescence detector
14 : Back pressure regulator

Fig.2 Schematic diagram of the SFC-UV-FL.

**Rectangular tubular shape of conventional FL flow cell**




**Cylindrical shape of novel FL flow cell***

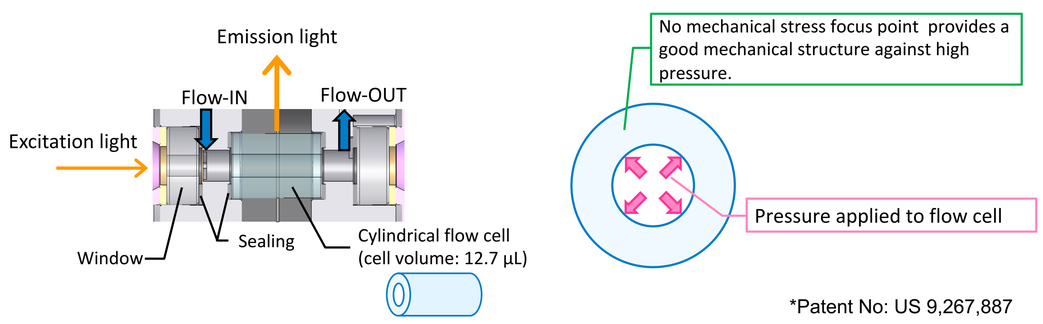
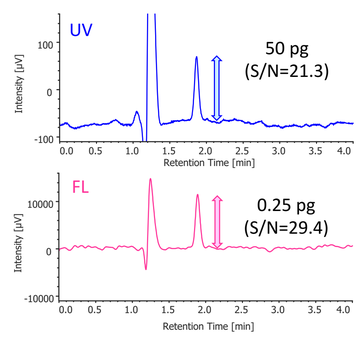



Fig.3 Structures and differences between the conventional and novel fluorescence flow cell.

## Results and Discussion

### SFC/UV vs. SFC/FL




| | Detection limit (S/N=3) |
|---|---|
| UV | 7040 fg |
| FL | 26 fg |

Approx. 300 times superior to UV.

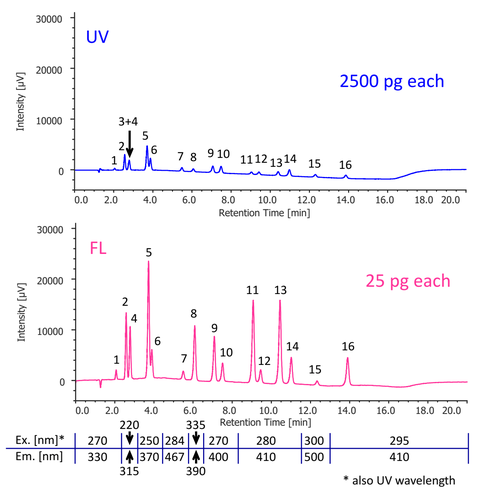
**[Analysis Conditions]**

| | |
|---|---|
| Column : | SFCpak DIOL-5 (Jasco) (4.6 mmI.D. x 250 mmL, 5 µm) |
| Mobile phase : | $CO_2$ / methanol (90 / 10) |
| Flow rate : | 3.0 mL/min |
| Column temp.: | 40 ºC |
| UV detection : | 250 nm |
| FL detection : | Ex. 250 nm, Em. 402 nm |
| Back pressure : | 15 MPa |
| Injection volume : | 5 µL |

Fig.4 Sensitivity of anthracene by SFC/UV and SFC/FL.

### SFC/FL Applications

**Polycyclic Aromatic Hydrocarbons (PAH)**




FL detection limits : 0.17-4.7 pg (20-400 times superior to UV)

**[Analysis Conditions]**

| | |
|---|---|
| Column : | 2-Ethylpyridine (Princeton) (4.6 mmI.D. x 250 mmL, 5 µm) |
| Mobile phase : | $CO_2$ / acetonitrile (gradient elution) |
| Flow rate : | 3.0 mL/min |
| Column temp.: | 40 ºC |
| Back pressure : | 15 MPa |
| Injection volume : | 5 µL |
| Sample : | 16 mixture of PAH in acetonitrile |

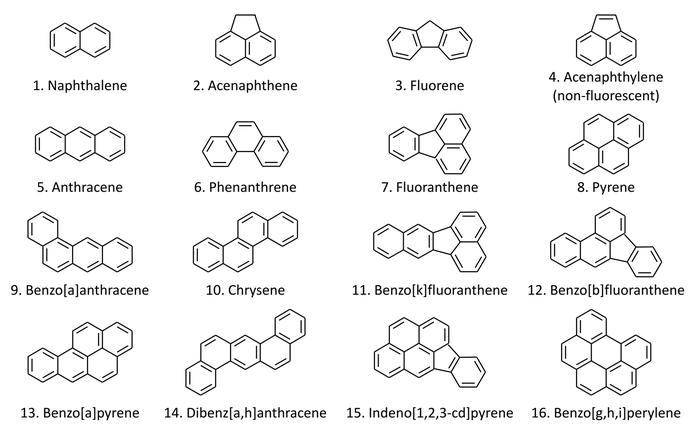
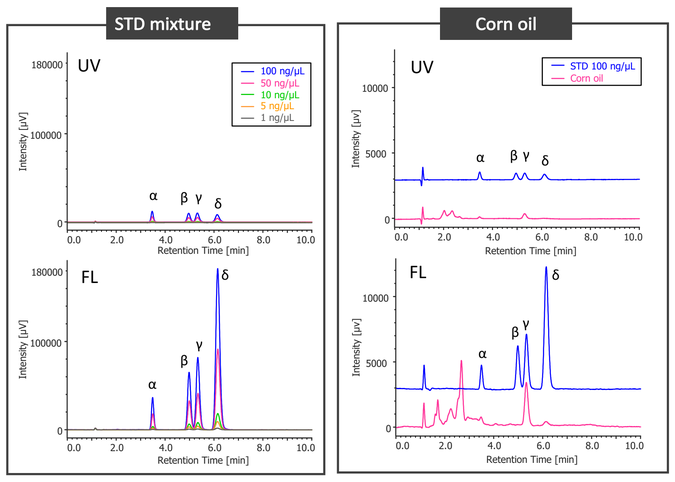


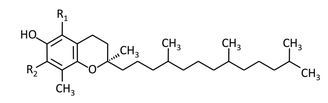

Fig.5 Measurement results of 16 mixture of PAH by SFC/UV and SFC/FL.

**Vitamin E (α, β, γ, δ-Tocopherol)**




**[Analysis Conditions]**

| | |
|---|---|
| Column : | SFCpak NH2-5 (Jasco) (4.6 mmI.D. x 250 mmL, 5 µm) |
| Mobile phase : | $CO_2$ / ethanol (93 / 7) |
| Flow rate : | 3.0 mL/min |
| Column temp.: | 40 ºC |
| UV detection : | 295 nm |
| FL detection : | Ex. 295 nm, Em. 330 nm |
| Back pressure : | 15 MPa |
| Injection volume : | 5 µL |
| Sample : | ・Isomer mixture of tocopherol in hexane/ethanol (90/10)<br>・Corn oil 10 mg/mL in hexane/ethanol (90/10) (filtration with 0.45-µm membrane) |

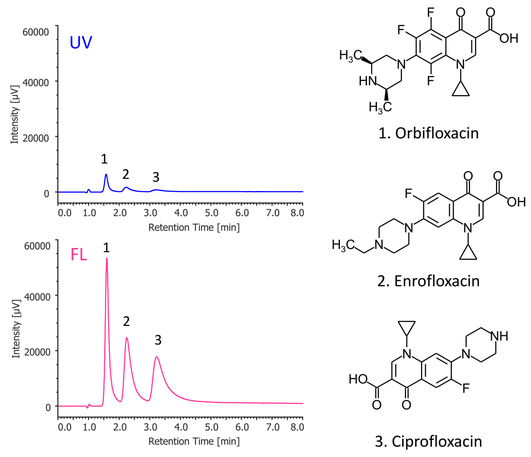


| | $R_1$ | $R_2$ |
|---|---|---|
| α- | $CH_3$ | $CH_3$ |
| β- | $CH_3$ | H |
| γ- | H | $CH_3$ |
| δ- | H | H |

Fig.6 Measurement results of isomer mixture of tocopherol by SFC/UV and SFC/FL.

FL detection limits : 0.19-0.96 ng (2-5 times superior to UV except for α-tocopherol)

**Quinolone Antibiotic**




**[Analysis Conditions]**

| | |
|---|---|
| Column : | SFCpak C18T-5 (Jasco) (4.6 mmI.D. x 250 mmL, 5 µm) |
| Mobile phase : | $CO_2$ / methanol with 100mM ammonium acetate and 0.1% diethylamine (gradient elution) |
| Flow rate : | 3.0 mL/mn |
| Column temp.: | 40 ºC |
| UV detection : | 290 nm |
| FL detection : | Ex. 290 nm, Em. 450 nm |
| Back pressure : | 15 MPa |
| Injection volume : | 5 µL |
| Sample : | 3 mixture of quinolone antibiotics (Orbifloxacin, enrofloxacin, and ciprofloxacin each 5 pg/µL in methanol) |

FL detection limits : 25-122 pg (4-14 times superior to UV)

Fig.7 Measurement results of 3 mixture of quinolone antibiotics by SFC/UV and SFC/FL.

## Conclusion

- We developed a fluorescence detector with a flow cell specifically constructed for operation at the high pressures required for SFC
- SFC/FL provides highly sensitive detection (from fg to pg) for fluorescent substances, compared to UV/Visible detector.
- SFC using fluorescence detection will open the door to new applications fields.

## References

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