A novel approach to cleaning validation for pharmaceutical manufacturing by online supercritical fluid extraction/supercritical fluid chromatography

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1. Introduction

Cleaning validation is necessary to establish the quality and safety of pharmaceutical drug products. In cleaning validation protocols, direct sampling is performed with swabs, which are sticks with textiles at one end. The sample on the swab after swabbing the surface of equipment is analyzed with a TOC analyzer and HPLC. Recently, HPLC has been more preferable because of the growing need for the individual analysis of products. Before the HPLC analysis, manual processes such as a sample extraction and a sample condensation are required. Such manual processes may affect to the quality of results. Thus, we evaluated the application of a novel on-line supercritical fluid extraction/chromatography system for the cleaning validation.

2. Experimental

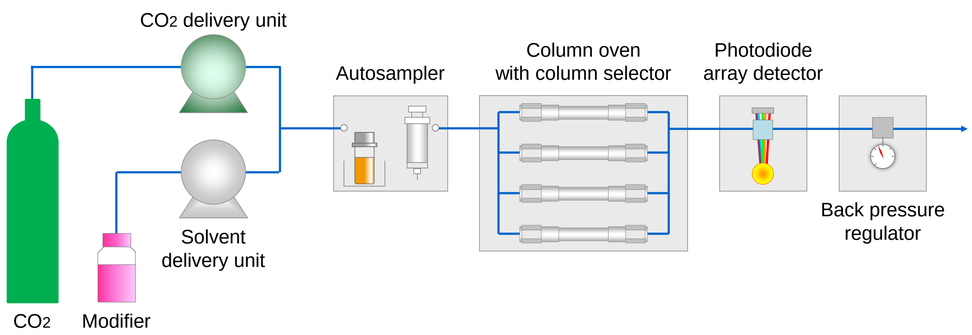
Materials and Reagents

A commercially available detergent containing alkylbenzene sulfonates was diluted with methanol and used as a standard sample. For the test of sample extraction, the sample was dropped onto a swab (ITW Texwipe, USA).

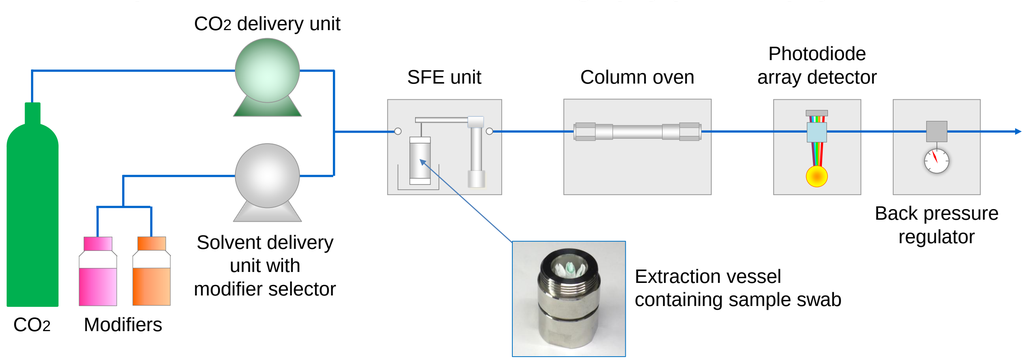
System

Nexera UC system (Shimadzu corporation, Japan) was used for both the screening of the method using supercritical fluid chromatography (SFC) (Figure 1A) and the supercritical fluid sample extraction (SFE) followed by SFC directly (SFE/SFC) (Figure 1B). The schematic diagram for static and dynamic extraction of supercritical fluid extraction unit is shown in Figure 1C.

1A: Supercritical fluid chromatography (SFC) system for analytical method development



1B: On-line Supercritical fluid extraction/chromatography (SFE/SFC) system



1C: Static and Dynamic extraction

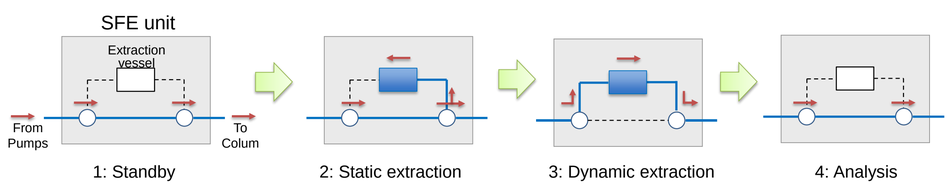


Figure 1. Flow diagrams of Nexera UC system

Analytical conditions

Table 1 SFC Analytical conditions

Column	:	Shim-pack UCX series columns (250 mm L. x 4.6 mm I.D., 5 µm) (I) UCX-RP (ODS with polar group), (II) UCX-GIS (ODS), (III) UCX-SIL, (IV) UCX-DIOL
Mobile Phase	:	A: CO_2; B: Methanol
Time program	:	Shown in the figure
Flow Rate	:	3.0 mL/min
Column Temp.	:	40°C
Back pressure	:	15 MPa
Wavelength	:	220 nm
Injection Vol.	:	Shown in figure

Table 2 On-line SFE/SFC Analytical conditions

[Sample Preparatation]
A total of 10 to 500 µg standard samples in methanol were dropped onto a total of three swabs.
The swabs were enclosed into an extraction vessel and set to the SFE/SFC unit.

[Static extraction]		
Extraction time	:	3 min
Mobile phase	:	A: CO_2; B: 0.1% (w/v) ammonium formate in methanol
B conc.	:	10%
Flow rate	:	3.0 mL/min
Back pressure	:	15 MPa
[Dynamic extraction]		
Extraction time	:	3 min
Mobile phase	:	A: CO_2; B: Methanol
B Conc.	:	10%
Flow rate	:	3.0 mL/min
Back pressure	:	15 MPa
[SFC]		
Column	:	Shim-pack UCX-SIL (250 mm L. x 4.6 mm I.D., 5 µm)
Mobile Phase	:	A: CO_2; B: Methanol
Time program	:	10%B (0-2 min), 10-60%B (2-7 min), 60%B (7-9 min), 10%B (9-13 min)
Flow Rate	:	3.0 mL/min
Column Temp.	:	40°C
Back pressure	:	15 MPa
Wavelength	:	220 nm

3. Results

Analytical method development

The analytical method for SFC was developed by screening four columns and gradient conditions. The UCX-SIL column showed excellent peak shape (Figure 2) and was used for further analysis.

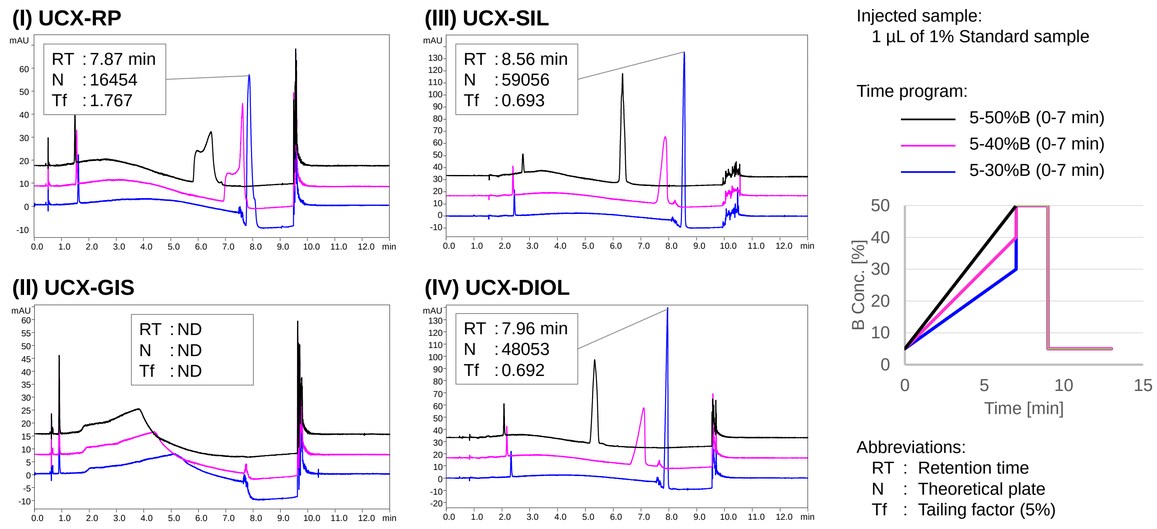
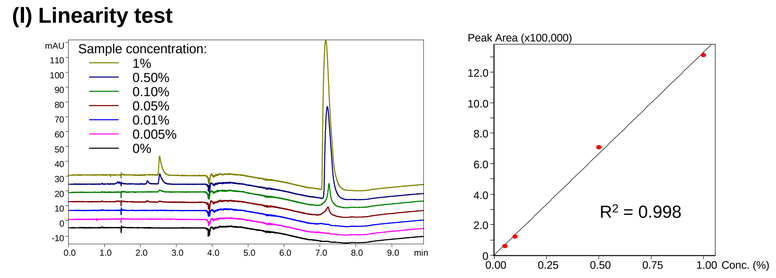


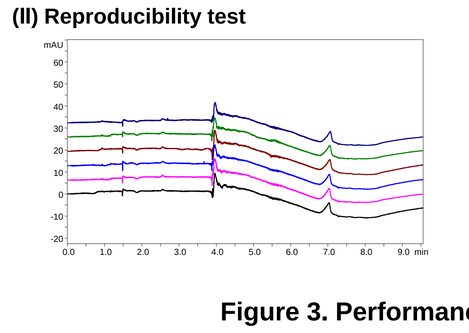
Figure 2. Comparison of columns for SFC analysis of standard detergent sample

The time program was developed in consideration of on-line SFE/SFC (data not shown). The optimized time program and the result of performance evaluations were shown in Figure 3.

(I) Linearity test



(II) Reproducibility test



Sample	Reproducibilities (n=6, %RSD)	
	Retention time	Area
0.10% standard sample	0.17	2.46

Injected sample:
2 µL of 0 to 1% Standard samples

Time program:
10%B (0-2 min), 10-60%B (2-7 min), 10%B (9-13 min)

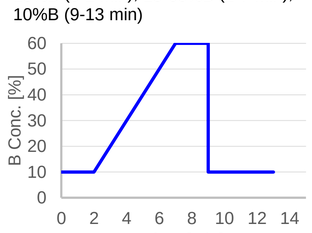
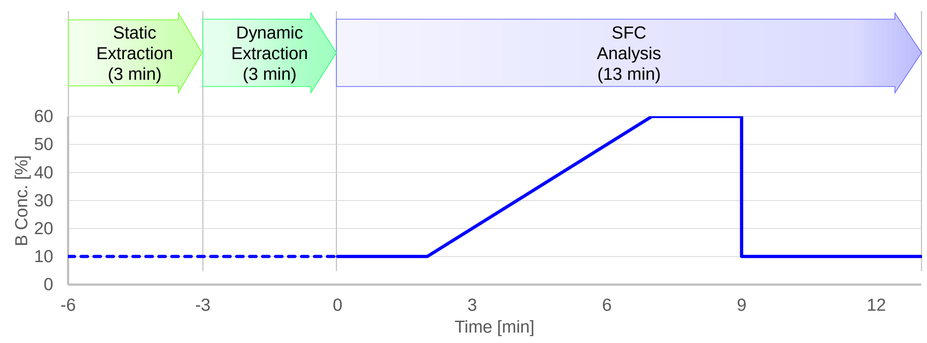


Figure 3. Performance evaluations of SFC analytical method

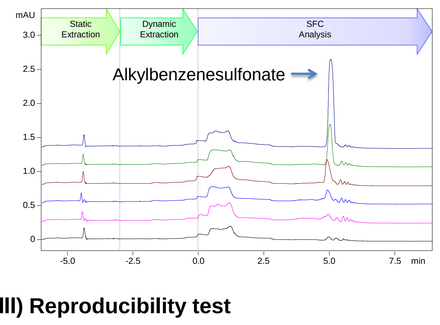
On-line SFE/SFC analysis of detergent in swab

Based on the optimized SFC analytical condition, an on-line SFE/SFC analytical condition was developed and its performance was evaluated (Figure 4).

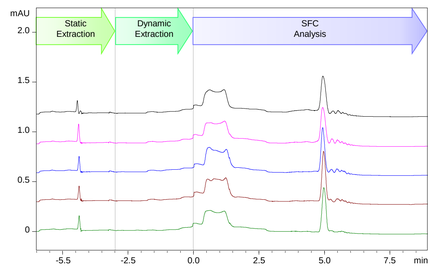
(I) Sequential diagram of on-line SFE/SFC



(II) Linearity test



(III) Reproducibility test



Sample	Reproducibilities (n=5, %RSD)	
	Retention time	Area
100 µg standard sample	0.19	5.76

Figure 4. On-line SFE/SFC analysis of standard detergent sample-spiked swabs

4. Conclusion

The evaluation results showed that the on-line SFE/SFC can provide reliable data for the cleaning validation for pharmaceutical manufacturing. The benefit of on-line SFE/SFC analysis is not only data reliability but also the convenience and safety gained by eliminating the manual extraction process. It may streamline laboratory processes and improve productivity and safety of pharmaceutical manufacturing facilities.

5. Acknowledgement

The sample was provided by DAIICHI SANKYO COMPANY, LIMITED.