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An evaluation of rapid method for simultaneous analysis of ciclesonide and its impurities in an inhaler using online SFE-SFC-QTOFMS

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1. Overview

In this study, we investigated a rapid qualitative analytical method of an active pharmaceutical ingredient, related impurities and impurities delivered from the container in a metered-dose inhaler. Nexera™ UC and LCMS™-9030 Quadrupole TOF mass spectrometer were used for online SFE-SFC-QTOFMS analysis.

2. Introduction

The efficacy and safety of pharmaceutical products are ensured by their quality. Since it is essential that the quality of drug substances and pharmaceutical products are confirmed by analyzing the impurities present, high-throughput analysis method is very useful for these analyses. We attempted to establish a rapid online SFE-SFC-QTOFMS method for simultaneous analysis of active pharmaceutical ingredients and their impurities using ciclesonide metered-dose inhaler (CIC-MDI) approved for the treatment of bronchial asthma as Alvesco®. Reduction of the sample preparation time associated with manual procedures has been accomplished by an innovative new concept of online SFE-SFC. Furthermore, the qualitative information of impurities was obtained by using HRMS by QTOFMS.

3. Methods

Alvesco Inhaler was purchased from Teijin Pharma Ltd. (Tokyo, Japan). Ciclesonide and its impurities were purchased from other reagent companies. The mixed standard solution was composed of ciclesonide, four impurities (Impurity A, B, C, IF1) listed in the European Pharmacopoeia(EP) or in supplement of package inserts for Alvesco also known as “Pharmaceutical interview forms“ (IF) in Japan, and the other 2 possible impurities (BT, MBT) derived from the container. Figure 1 shows the chemical structures of ciclesonide, impurities listed in EP or IF and other potential impurities in the inhaler.

The glass disc was attached to the inlet port of the inhaler and sprayed once. A punched disc or a disc spotted with standard impurities was placed in an extraction vessel (0.2 mL) for SFE.

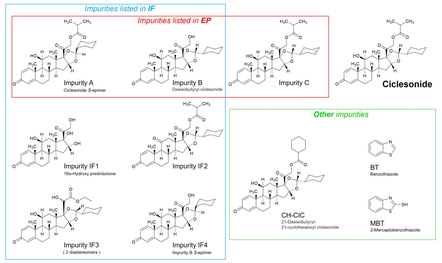

Figure 1 Chemical structures of ciclesonide and its impurities.

After extracting the sample matrix with SFE for 3.5 minutes, the valve was automatically switched to on-column SFC-QTOFMS analysis mode for 6.5 minutes without any human intervention. (Figure 2, Nexera UC with LCMS-9030, Shimadzu Corporation, Kyoto, Japan). The mobile phases used were (A) supercritical fluid of carbon dioxide and (B) methanol containing 10 mmol/L ammonium formate. SFC-MS with electrospray ionization was operated in positive scan mode.

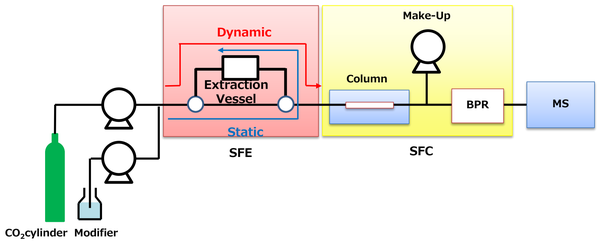

Figure 2 Schematic flow diagram of online SFE-SFC-QTOFMS System

Analytical Conditions

SFE conditions (Nexera UC system)

Modifier	: 10 mM Ammonium formate in MeOH
Flow rate	: 3 mL/min
Extraction	: 0-0.5 min Static extraction ＊10% of modifier 0.5-3.5 min Dynamic extraction ＊10% of modifier
Extraction temp.	: 60 ˚C
BPR pressure	: 10 MPa
Vessel Volume	: 0.2 mL

SFC conditions (Nexera UC system)

Column	: CHIRALPAK IE-3 (100 mm×3.0mmI.D., 3 μm)
Modifier	: 10 mM Ammonium formate in MeOH
Flow rate	: 3 mL/min
Gradient	: 0.5% (3.5-4 min) → 25% (4.1 min) → 40% (6-8 min) → 100% (8.1-9.5 min) → 0.5% (9.5-10 min)
BPR pressure	: 10 MPa
Column temp.	: 40 ˚C

MS conditions (LCMS-9030)

Ionization	: ESI, Positive Scan mode
Make-up	: MeOH
Make-up Flow rate	: 0.1 mL/min
Nebulizing Gas Flow	: 3.0 L/min
Heating gas Flow	: 10.0 L/min
Drying Gas Flow	: 10.0 L/min
Interface Temp.	: 300 ˚C
DL Temp.	: 250 ˚C
Heat Brock Temp.	: 400 ˚C
Probe Position	: +1 mm
Scan Range	: *m/z* 100-1000

4. Results

4-1. Column scouting to separate ciclesonide and its impurities

Column scouting was conducted before SFE-SFC-QTOFMS analysis for CIC-MDI. SFC columns with different stationary phases exhibited significant differences in both selectivity and polarity. To simultaneously determine ciclesonide and its impurities as narrow and symmetrical peaks, 20 different SFC columns (Shim-pack™ UC Diol, RP, Sil, NH2, CN, phenyl, amide, GIS II, Shimadzu corporation / CHIRALPAK® AD-3, OX-3, AY-3, OD-3, OJ-3, OZ-3, IA-3, IB-3, IC-3, ID-3, IE-3, IF-3, Daicel corporation Osaka, Japan) were evaluated with a mixed standard solution by SFC-QTOFMS. Figure 3 shows the respective SFC-MS chromatograms on analytes using four different columns. The IE-3 column presented the better resolution of ciclesonide and these impurities than others, with all the peaks exhibiting satisfactory separation effects and peak shapes.

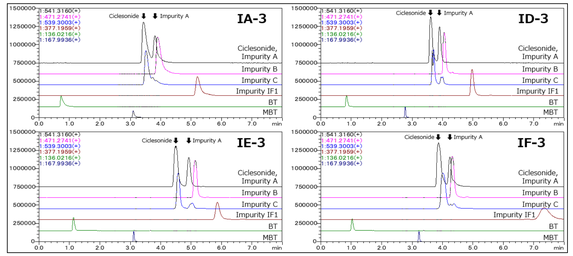

Figure 3 Comparison of the separation effects of extracted ion chromatogram of SFC-QTOFMS among the four different SFC columns.

4-2. Online SFE-SFC-QTOFMS Analysis

The mixed standard solution was analyzed by online SFE-SFC-QTOFMS with extraction solvents consisting of liquid carbon dioxide/methanol containing 10 mM ammonium formate (90:10) under static extraction for 0.5 min and dynamic extraction conditions for 3 min at 60 ˚C. These compounds were extracted well in 3.5 min and were separated with SFC-MS by modifying the condition of gradient elution. Figure 4 shows the SFE-SFC QTOFMS chromatograms of ciclesonide and its impurity standards.

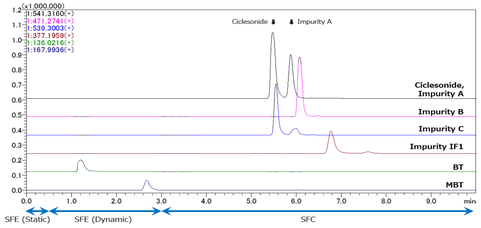

Figure 4 Extracted ion chromatograms of an online SFE-SFC-QTOFMS of ciclesonide and its impurities.

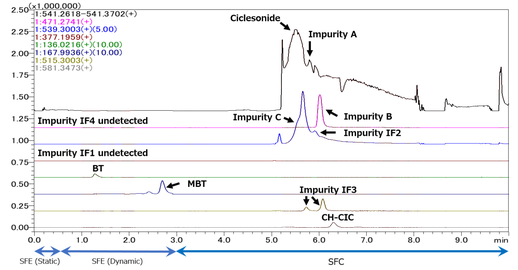

Figure 5 Extracted ion chromatogram of an online SFE-SFC-QTOFMS of ciclesonide and the other impurities metered-dose inhaler.

The CIC-MDI was analyzed by online SFE-SFC-QTOFMS developed. A one-pushed sample on the glass disc was analyzed with quite high mass accuracy in 10 min including extraction time. Extracted ion chromatogram of CIC-MDI compounds are shown in Figure 5.

The mass value of detected compounds are summarized in Table 1. Ciclesonide, impurity A, impurity B, impurity C, BT and MBT were identified by comparing retention time and accurate mass of their respective standards. At this time, impurity C was detected at trace level. Furthermore, Impurity IF2, Impurity IF3 (2 diastereomers) and CH-CIC were tentatively identified from their accurate mass without their respective standards. Impurity IF1 and impurity IF4 were not detected.

Table 1 The detected compounds by online SFE-SFC-QTOFMS

Compound	Formula and Theoretical *m/z* $[M+H]^+$	Observed *m/z*	error (mDa)	RT	Listed in EP	Listed in IF
Ciclesonide	$C_{32}H_{44}O_7$, *m/z* 541.3160	541.3159	-0.1	5.48	✓	✓
Impurity A	$C_{32}H_{44}O_7$, *m/z* 541.3160	541.3158	-0.2	5.87	✓	✓
Impurity B	$C_{28}H_{38}O_6$, *m/z* 471.2741	471.2735	-0.6	6.06	✓	✓
Impurity C	$C_{32}H_{42}O_7$, *m/z* 539.3003	539.2993	-1.0	5.54	✓	
Impurity IF2	$C_{32}H_{42}O_7$, *m/z* 539.3003	539.2995	-0.8	5.96		✓
Impurity IF3 (2 diastereomers)	$C_{30}H_{42}O_7$, *m/z* 515.3003	515.2998	-0.5	5.74		✓
		515.3000	-0.3	6.08		✓
CH-CIC	$C_{35}H_{48}O_7$, *m/z* 581.3473	581.3463	-1.0	6.33		
BT	C_7H_5NS, *m/z* 136.0216	136.0211	-0.5	1.27		
MBT	$C_7H_5NS_2$, *m/z* 167.9936	167.9932	-0.4	2.70		

5. Conclusions

- We developed the screening analytical method for the active pharmaceutical ingredients and impurities in metered-dose inhaler using the online SFE-SFC-QTOFMS.
- The compounds were detected with quite high mass accuracy within 10 minutes including extraction time.
- Sample preparation was only spraying by using an online SFE-SFC-QTOFMS system.
- We confirmed that these rapid method using an online SFE-SFC-QTOFMS was a very powerful tool for profiling of impurities on developing new pharmaceutical products.

6. Reference

[1] Seiji Tanaka et al., Journal of Pharmaceutical and Biomedical Analysis Volume 204, 10 September 2021, 114253, doi:10.1016/j.jpba.2021.114253.