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Application News

High Performance Liquid Chromatograph Mass Spectrometer LCMS™-9030

Fast Analysis of Ciclesonide Inhalant by Online SFE-SFC-QTOFMS

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User Benefits

- Online SFE-SFC-QTOFMS can be used to quickly screen for impurities in pharmaceuticals.
- A wide variety of impurities can be analyzed simultaneously with almost no sample pretreatment.

■ Introduction

Checking the content of active ingredients and impurities is essential for maintaining pharmaceutical quality, so establishing a quick and convenient screening method would help shorten development periods and promote practical use of pharmaceuticals.

Online SFE-SFC-QTOFMS systems use a combination of supercritical fluid extraction (SFE) and supercritical fluid chromatography (SFC) for extracting components with supercritical fluid in an extraction vessel, injecting the extracted components into an SFC unit for online column separation, followed by accurate mass analysis of separated components in a quadrupole-time of flight (QTOF) mass spectrometer system. The online SFE-SFC system can also add a polar organic solvent to supercritical carbon dioxide fluid as a modifier to enable extraction and separation of components with a wide range of polarities.

This article describes an example of using the online SFE-SFC-QTOFMS method for screening analysis of active ingredients and impurities in the drug Alvesco, a ciclesonide inhalant used to treat bronchial asthma.

■ Screening Target Compounds and Online SFE-SFC-QTOFMS System

To determine the analytical conditions, a standard mixture sample was prepared from standard samples of ciclesonide, relevant impurities A, B, and C listed in the European Pharmacopoeia (EP), relevant impurity IF1 indicated in the Alvesco pharmaceutical interview form (IF), and impurities benzothiazole (BT) and 2-mercaptobenzothiazole (MBT) that potentially could be contained in vials. Fig. 1 shows the structural formulas for ciclesonide and the impurities.

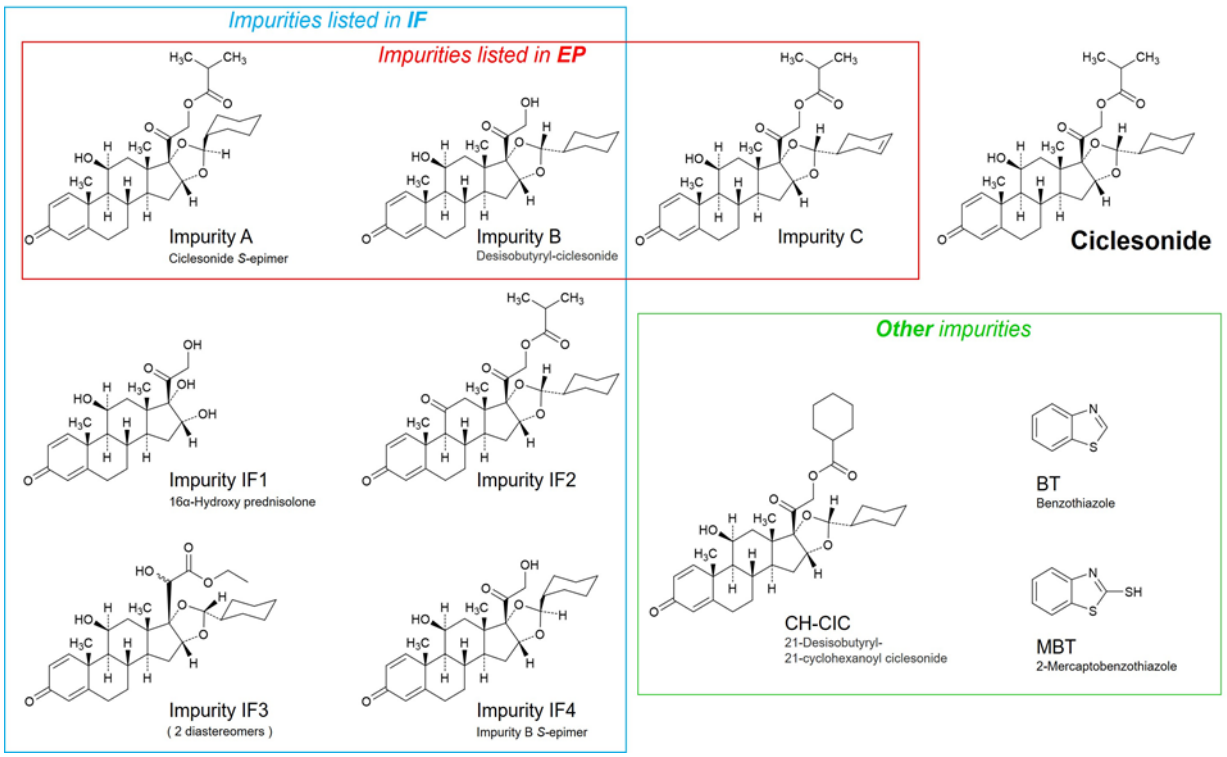

Fig. 1 Structural Formulas for Ciclesonide and Impurities

Fig. 2 Shows the configuration of the online SFE-SFC-QTOFMS system. There are two types of extraction. Static extraction adds supercritical fluid to the extraction vessel and then extracts components without pumping fluid through the unit, whereas dynamic extraction extracts components while pumping fluid through the unit. In the case of online SFE-SFC-QTOFMS, extracted substances enter the analytical column during dynamic extraction, separated by gradient elution, and then their masses are accurately measured by QTOFMS.

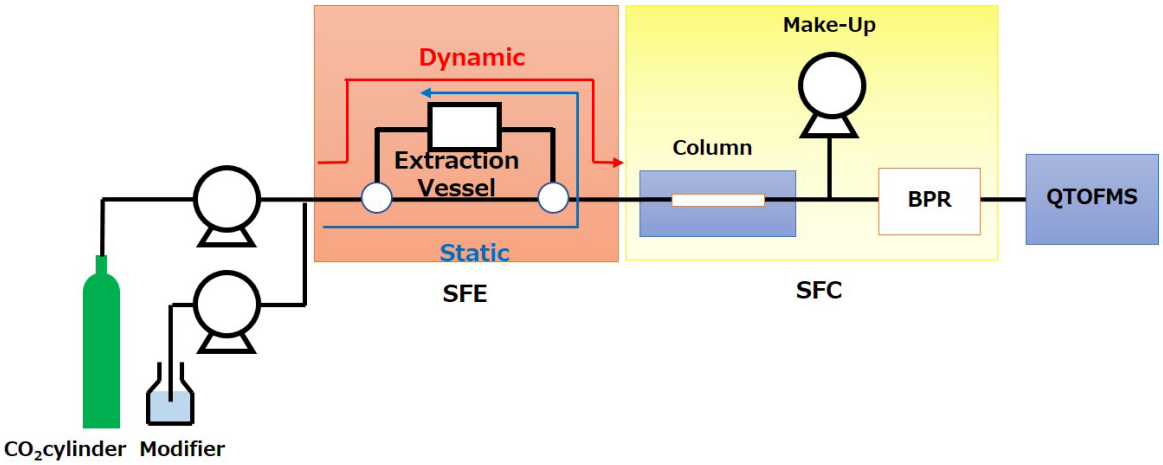

Fig. 2 Online SFE-SFC-QTOFMS System

Table 1 SFE-SFC Analytical Conditions (Nexera™ UC)

[SFE]	
Solvent:	A) CO_2
	B) 10 mM Ammonium formate in methanol
Flowrate:	3 mL/min
Extraction:	0-0.5 min Static mode (B. Conc. 10 %)
	0.5-3.5 min Dynamic mode (B. Conc. 10 %)
Extraction Temp.:	60 °C
BPR Pressure:	10 MPa
Vessel Volume:	0.2 mL
[SFC]	
Column:	CHIRALPAK IE-3
	(100 mm × 3.0 mm I.D., 3 mm)
Mobile Phase:	A) CO_2
	B) 10 mM Ammonium formate in methanol
Flowrate:	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

Table 2 MS Analytical Conditions (LCMS-9030)

Ionization:	ESI (Positive scan mode)
Make-up:	Methanol
Make-up Flowrate:	0.1 mL/min
Nebulizing Gas Flow:	3.0 L/min
Heating Gas Flow:	10 L/min
Drying Gas Flow:	10 L/min
Interface Temp.:	300 °C
DL Temp.:	250 °C
Block Heater Temp.:	400 °C
Scan Range:	*m/z* 100-1000

■ Determining SFC Separation Parameters by Column Scouting

The standard mixture sample was used to determine SFC separation parameters by column scouting. A total of 20 columns were evaluated by column scouting, namely Shimadzu Shim-pack™ UC columns (Diol, RP, Sil, NH2, CN, Phenyl, Amide, and GIS II) and Daicel CHIRALPAK SFC chiral analysis columns (AD-3, OX-3, AY-3, OD-3, OJ-3, OZ-3, IA-3, IB-3, IC-3, ID-3, IE-3, and IF-3).

Fig. 3 shows extracted ion chromatograms from using four representative columns. It was decided that the CHIRALPAK IE-3 column with a stationary phase of amylose tris (3, 5-dichlorophenylcarbamate) performed the best in terms of separating ciclesonide and the impurities and providing adequate separation and good peak shape.

■ Online SFE-SFC-QTOFMS Analysis

The standard mixture sample dripped onto a glass disc was sealed inside the extraction vessel and measured by online SFE-SFC-QTOFMS in accordance with the analytical conditions indicated in Tables 1 and 2. Due to the simple sample preparation process, all seven compounds were extracted, separated by SFC, and their masses accurately measured within 10 minutes. Fig. 4 shows the extracted ion chromatograms obtained from the standard mixture sample.

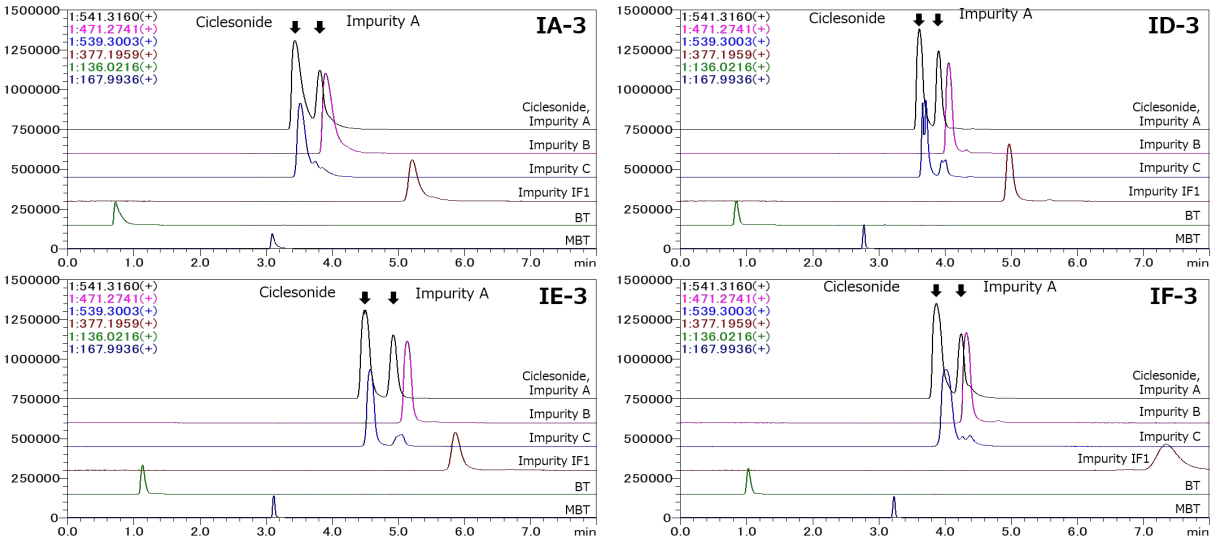

Fig. 3 Examples of SFC Separation Using Representative Columns

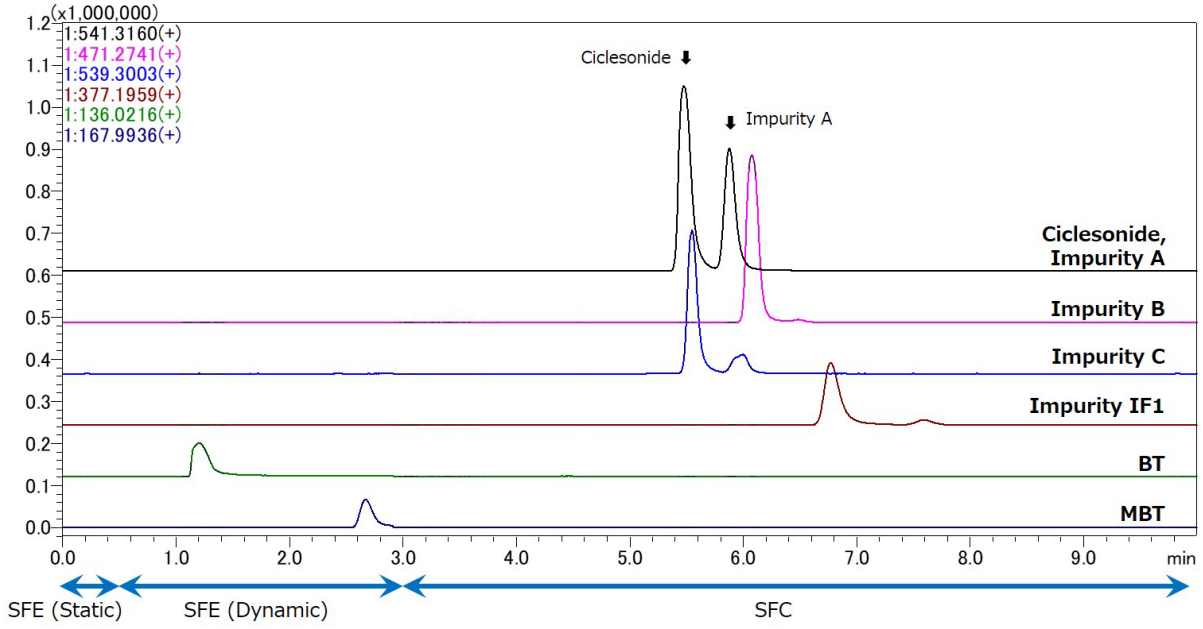

Fig. 4 Extracted Ion Chromatograms from Standard Mixture Sample

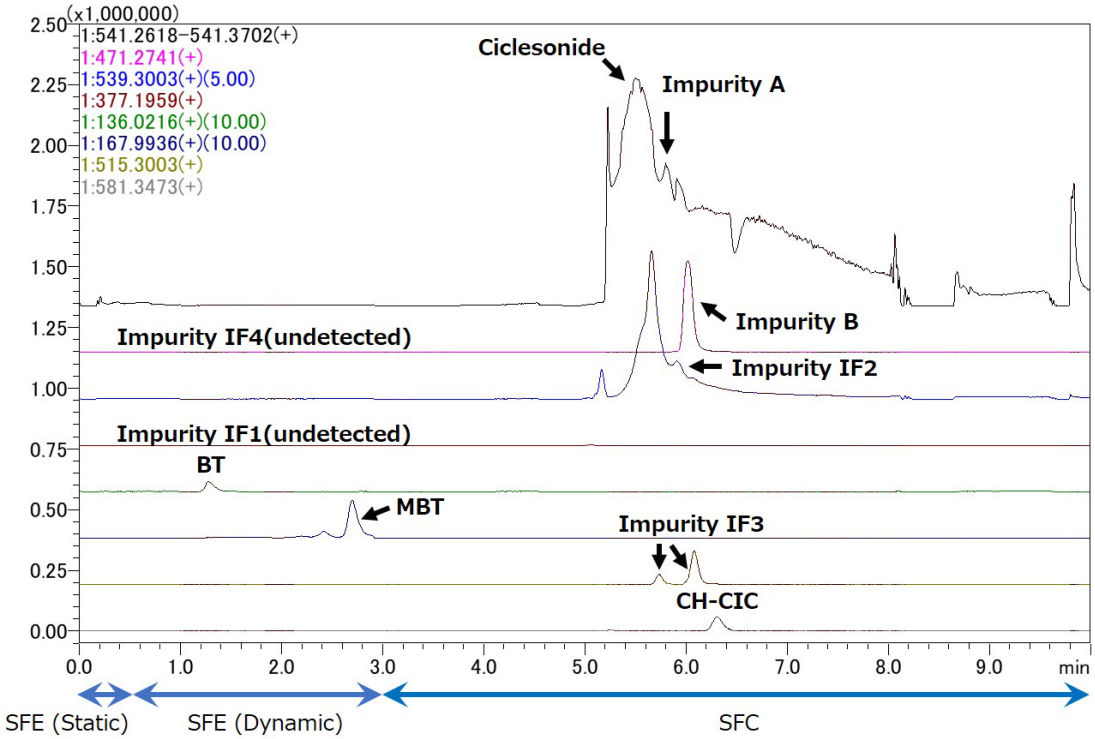

Fig. 5 Extracted Ion Chromatograms from Alvesco

Table 3 Compounds Detected from Alvesco by Online SFE-SFC-QTOFMS

Compound	Formula and Theoretical *m/z* ($[M+H]^+$)	Observed *m/z*	Error (mDa)	RT (min)	Listed in	
					EP	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		

The Alvesco sample sprayed once onto a glass disc was sealed inside the extraction vessel and measured by online SFE-SFC-QTOFMS.

Fig. 5 shows extracted ion chromatograms for Alvesco-related compounds and Table 3 lists the accurate masses of detected peaks. Ciclesonide, impurity A, impurity B, impurity C, BT, and MBT were identified based on retention times and accurate masses from the standard mixture sample. Impurity IF2, impurity IF3 (with two peaks presumably from diastereomer), and CH-CIC, that were included in the standard mixture sample, were identified based on accurate masses. Impurity IF1 and impurity IF4 were not detected.

■ Conclusion

A method for using an online SFE-SFC-QTOFMS system to quickly and easily screen for active ingredients and impurities in the drug Alvesco was established. The method is also expected to contribute to profiling impurities during new drug development.

This research was conducted in partnership with the National Institute of Health Sciences.

[Ref] Tanaka S., et al., J. Pharm. Biomed. Anal. 204, 114253 (2021), doi.org/10.1016/j.jpba.2021.114253

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