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Structural analysis of impurities in pharmaceutical ingredients using trap-free 2D-LC high-resolution accurate mass spectrometry

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Overview

Structural analysis of trace impurities separated with non-volatile mobile phase was performed by using a trap-free 2D-LC QTOF system.

Introduction

High performance liquid chromatography (HPLC) is the primary means for impurity testing in pharmacopeia. Solutions containing non-volatile salts are frequently used as a mobile phase because of the irreplaceable advantage to achieve better chromatographic performances. However, non-volatile mobile phases are not compatible with mass spectrometry which is commonly used for structural analysis of impurities. Trap-free two-dimensional HPLC (trap-free 2D-LC) can fill this gap by exchanging a non-volatile mobile phase with a volatile one compatible for mass spectrometry. In this study, we demonstrate how structural analysis of impurities in pharmaceutical ingredients is feasible by using trap-free 2D-LC coupled to quadrupole time-of-flight mass spectrometry (QTOF MS) without modifying the original method mobile phases.

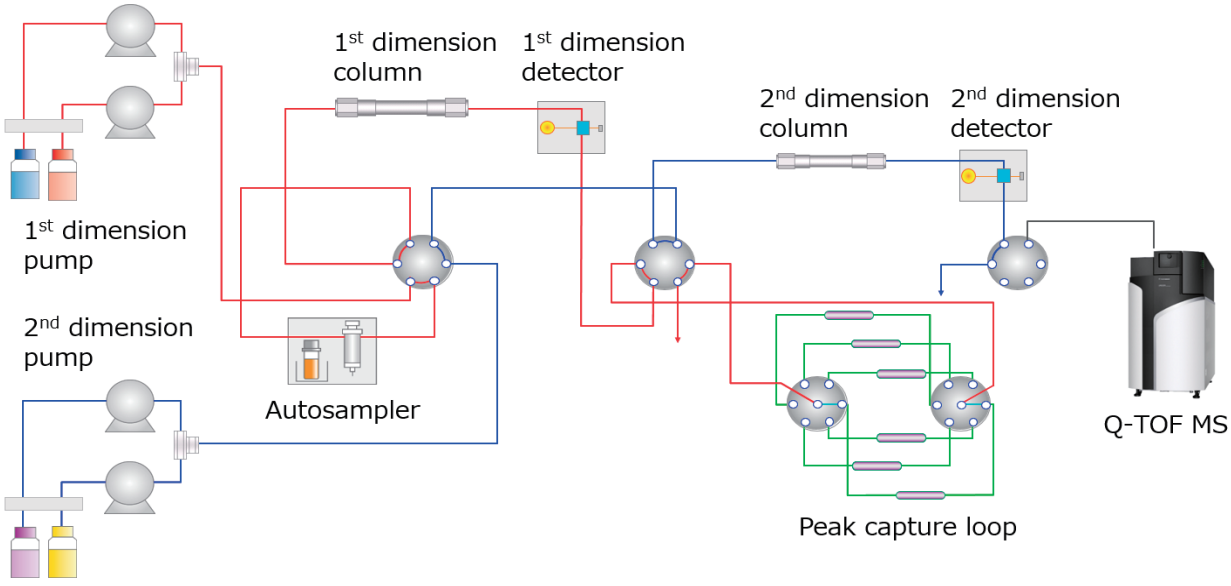

Figure 1 Flow line diagram of trap-free 2D-LC

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Methods

Trap-free 2D-LC impurity analysis system coupled with a LCMS -9030 (Shimadzu Corporation, Japan) was used in this study. The flow line diagram is shown in Figure1. The flow of the mobile phases diverts with the valve positions in each operation. The flow line for the nonvolatile mobile phase is shown in red. The flow line for the volatile mobile phase is shown in blue. The peak capture loop for the fractionated impurities is shown in green. A test solution of atorvastatin calcium hydrate 1 mg/mL containing impurities was prepared using a commercially available laboratory reagent (Figure 2). The sample was then analyzed by the HPLC method with UV detection under non-volatile mobile phase condition described in the Japanese Pharmacopoeia, as shown in Table 1 (1st dimension). The impurities were fractionated in the peak capture loops and flushed out of the loop with the MS-compatible volatile mobile phase by switching the valve position and activation of the second dimension pump to introduce each peak in the MS (2nd dimension, Table 2).

Table 1 Analytical conditions (1st Dimension, HPLC-UV, non-volatile mobile phase)

Column	: Shim-pack™ VP-ODS (250 mmL. X 4.6 mmI.D., 5 µm)
Mobile Phase A	: Citrate buffer pH 5.0 / Acetonitrile / Tetrahydrofuran (4/1/1 = v/v/v)
Mobile Phase B	: Acetonitrile / Tetrahydrofuran (1/1 = v/v)
Time program	: B.Conc 7% (0-40 min) → 40% (80 min) → 7% (80.1-100 min)
Flow Rate	: 1.43 mL/min
Column Temp.	: 40 °C
Injection Volume	: 20 µL
Detection	: UV 254 nm

Table 2 Analytical conditions (2nd Dimension, QTOF MS, volatile mobile phase)

Column	: Shim-pack XR-ODS (50 mmL. X 2.0 mmI.D., 2.2 µm)
Mobile Phase A	: 10 mmol/L Ammonium Formate – water
Mobile Phase B	: Acetonitrile
Time program	: B.Conc 10% (0 min) → 100% (6-6.5 min) → 10% (6.51-10 min)
Flow Rate	: 0.3 mL/min.
Column Temp.	: 40 °C
Injection Volume	: 20 µL (Loop Volume)
Detection	: UV 254 nm, MS, MS/MS scan (ESI positive or negative Mode)

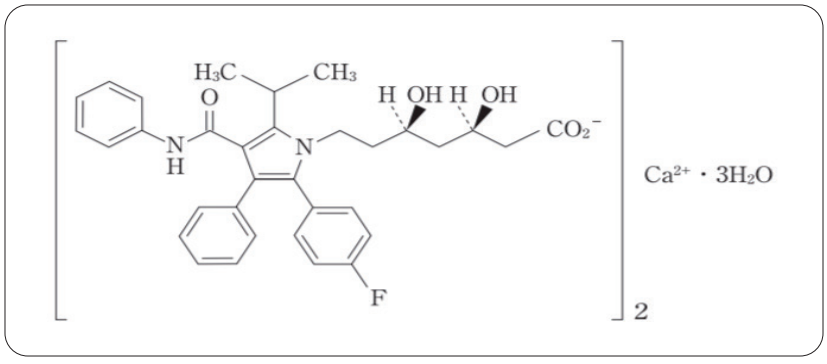

Figure 2 Structure of atorvastatin calcium hydrate

Results

HPLC Analysis via a Non-volatile Mobile Phase (1st Dimension)

In the 1st dimension analysis, atorvastatin calcium solution was analyzed by HPLC method with UV detection at a wavelength of 254 nm under non-volatile citrate buffer condition. As shown in Figure 3, atorvastatin, the main component, was eluted from the 1st dimension column at a retention time of about 16 minutes, and multiple impurity peaks were also observed in the chromatogram. Among them, the 16 impurities (including the main component) shown in the figure were fractionated into peak capture loops.

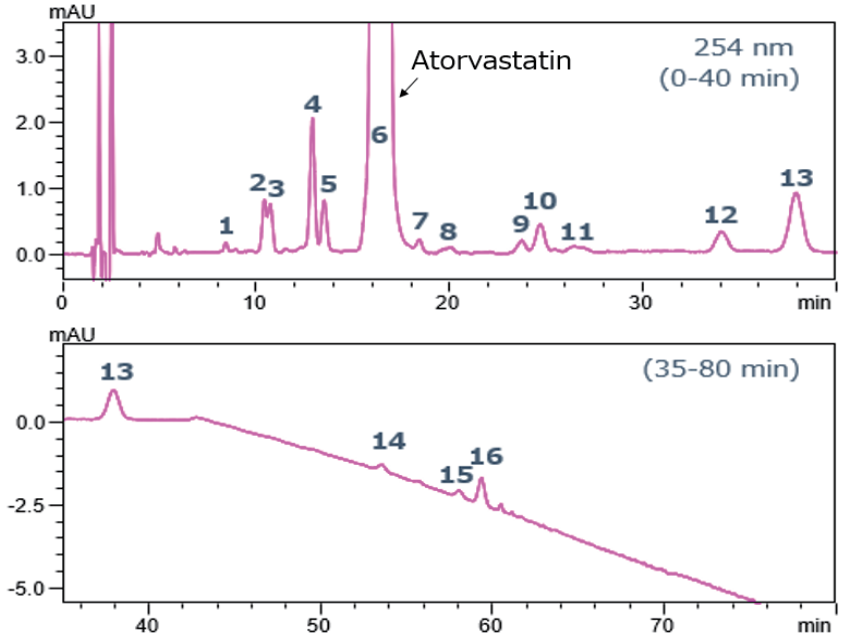

Figure 3 UV Chromatogram for atorvastatin calcium (1st Dimension)

QTOF MS analysis via a Volatile Mobile Phase (2nd Dimension)

In the 2nd dimension, the components in peak capture loops were automatically analyzed with QTOF MS under the volatile mobile phase condition. By comparing the results of blank and sample analysis, it is possible to clearly confirm the elution of impurity components from the second dimension column. The mass value of impurities in positive mode and negative mode are summarized in Tables 3 and 4. Atorvastatin and impurities described in European Pharmacopoeia (EP) were attributed with high mass accuracy within almost 1ppm compared to the theoretical mass value in both positive and negative ESI scan mode.

Table 3 Results of measurements of the Impurities (positive mode)

ID	RT (HPLC)	EP listed impurities	$[M+H]^+$ theoretical	$[M+H]^+$ observed	error (PPM)
1	8.44			591.2501	
2	10.44	impurity F	718.3498	718.3497	-0.13
3	10.74			575.2551	
4	12.92			575.256	
5	13.53	impurity A	541.2697	541.2695	-0.37
5	13.53			557.2445	
6	16.30	Atorvastatin	559.2603	559.2601	-0.31
7	18.39			557.2446	
8	20.10			557.2445	
9	23.73			601.2709	
10	24.68	impurity G	573.2759	573.2762	0.48
11	26.43			591.2500	
12	34.07			573.2393	
13	37.93	impurity H	541.2497	541.2499	0.35
14	53.51			416.1655	
15	58.02	impurity D	432.1606	432.1607	0.32
15	58.02			362.1187	
16	59.34	impurity D	432.1606	432.1605	-0.15

Table 4 Results of measurements of the impurities (negative mode)

ID	RT (HPLC)	EP listed impurities	$[M-H]^-$ theoretical	$[M-H]^-$ observed	error (PPM)
1	8.44			589.2354	
2	10.44	impurity F	716.3353	716.3358	0.70
3	10.74			573.2406	
4	12.92			573.241	
5	13.53	impurity A	539.2551	539.2552	0.10
5	13.53			555.2301	
6	16.30	Atorvastatin	557.2457	557.2457	0
7	18.39			555.2305	
8	20.10			N.D	
9	23.73			599.2565	
10	24.68	impurity G	571.2614	N.D	
11	26.43			N.D	
12	34.07			N.D	
13	37.93	impurity H	539.2352	N.D	
14	53.51			414.1506	
15	58.02	impurity D	430.1460	N.D	
15	58.02			360.1035	
16	59.34	impurity D	430.1460	430.1455	-1.19

QTOF MS/MS analysis

It was possible to obtain not only the molecular weight information for the impurities, but also a structural analysis from the product ion information. For example, MS/MS analysis of atorvastatin impurity F described in EP was performed. As shown in Figure 4, cleavage site in the structural formula was automatically assigned with high mass accuracy within almost 1ppm using fragment ion information predicted by the dedicated ACD/MS Workbook Suite™ software (ACD/Labs, Canada).

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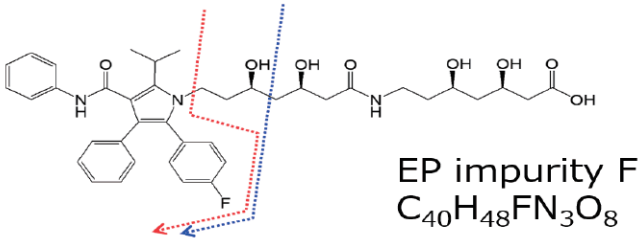

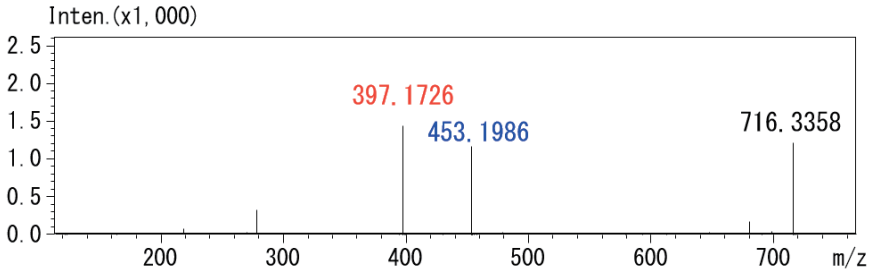

Predicted formula	theoretical mass	observed mass	error (PPM)
C26H22FN2O (-)	397.1722	397.1726	1.01
C29H26FN2O2 (-)	453.1984	453.1986	0.44
C40H47FN3O8 (-)	716.3353	716.3358	0.70

Figure 4 Results of an MS/MS analysis of impurity F described in EP

Conclusions

As shown here, it was possible to identify impurity peaks with high probability using non-volatile mobile phase conditions as is, through a combination of the LCMS-9030, which is capable of accurate MS/MS analysis, and a trap-free 2D HPLC.

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