

Application News

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LC/MS

Structural Analysis of Impurities in Pharmaceuticals Using Trap-Free 2D HPLC and the LCMS™-9030

Management and confirmation of the type, quantity, and safety of trace impurities contained in products are important points for ensuring the quality of products in a wide variety of fields including pharmaceuticals (raw materials, final pharmaceutical agents, and generic drugs), foods (health foods and supplements), and fine chemical products (solvents, paints, surfactants, and various chemical products). In terms of impurities in products, known component checks are performed via the HPLC-UV method in quality control departments. Conversely, the measurement and analysis of unknown components using mass spectrometers, which provide better sensitivity and excellent specificity, based on the HPLC-UV method, are requirements in research departments.

However, it is not possible to apply the nonvolatile mobile phase conditions used with HPLC as is in LC/MS analysis. Accordingly, in research departments, these are replaced by volatile mobile phase conditions, which is a very labor intensive process. These condition changes carry significant risks regarding changes to the elution order, and the omission of impurities eluted in the vicinity of main components, so very careful investigations are required.

This report introduces an example of the structural analysis in which impurities detected under nonvolatile mobile phase conditions were converted into volatile mobile phase conditions on-line without any complicated examination using a trap free 2-dimensional(2D) HPLC, and then subjected to precision mass spectrometry using the LCMS-9030 quadrupole time-of-flight mass spectrometer.

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■ HPLC Analysis via a Nonvolatile Mobile Phase (1st Dimension)

Here, a 1 mg/mL test solution was prepared using commercially available atorvastatin calcium as the test reagent. The measurements were performed based on the analysis conditions noted in the Japanese Pharmacopoeia, shown in Table 1. Because a nonvolatile citric acid buffer solution was used as the mobile phase, it could not be injected as is into the LC/MS.

Table 1 Analysis Conditions (1st Dimension)

| | |
|------------------|--|
| Column | : Shim-pack™ VP-ODS (250 mmL×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 |

The analysis was performed using a combination of the LCMS-9030 and a trap-free 2D HPLC, with the instrument configuration shown in Fig. 1. The flow of the mobile phases differs 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.

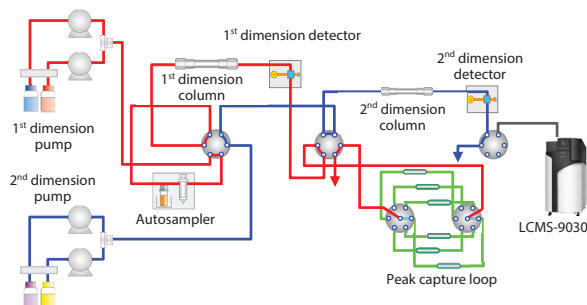


Fig. 1 Flow Line Diagram

The atorvastatin calcium solution was analyzed using this system, and the UV chromatogram obtained is shown in Fig. 2. Atorvastatin, the main component, was eluted with a retention time of approximately 16 minutes. Multiple impurity peaks can be confirmed in this vicinity. Of these, the 16 impurities (including the main component) shown in the figure were fractionated in the peak capture loop.

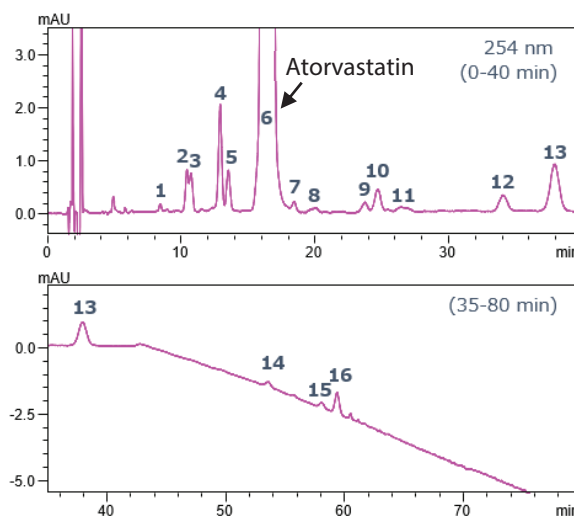


Fig. 2 UV Chromatogram for Atorvastatin Calcium (1st Dimension)

LC/MS Analysis via a Volatile Mobile Phase (2nd Dimension)

The main component and impurities (ID 1 to 16) fractionated in the peak capture loop were extruded from the loop using a volatile mobile phase via valve position switching and the 2nd dimensional solvent delivery unit, and were then injected into the LCMS-9030. The conditions used in the 2nd dimensional analysis are shown in Table 2.

Table 2 Analysis Conditions (2nd Dimension)

| | |
|------------------|--|
| Column | : Shim-pack XR-ODS (50 mmL.x2.0 mmL.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) |

Table 3 LCMS-9030 Measurement Results for the Impurities (Positive Mode)

| ID | RT (HPLC) | EP listed impurities | [M+H] ⁺ theoretical | [M+H] ⁺ observed | Error (PPM) | Content (%) |
|----|-----------|----------------------|--------------------------------|-----------------------------|-------------|-------------|
| 1 | 8.44 | | | 591.2501 | | 0.01 |
| 2 | 10.44 | Impurity F | 718.3498 | 718.3497 | -0.13 | 0.04 |
| 3 | 10.74 | | | 575.2551 | | 0.04 |
| 4 | 12.92 | | | 575.256 | | 0.12 |
| 5 | 13.53 | Impurity A | 541.2697 | 541.2695 | -0.37 | 0.05 |
| 5 | 13.53 | | | 557.2445 | | 0.05 |
| 6 | 16.30 | Atorvastatin | 559.2603 | 559.2601 | -0.31 | 99.41 |
| 7 | 18.39 | | | 557.2446 | | 0.01 |
| 8 | 20.10 | | | 557.2445 | | 0.01 |
| 9 | 23.73 | | | 601.2709 | | 0.01 |
| 10 | 24.68 | Impurity G | 573.2759 | 573.2762 | 0.48 | 0.03 |
| 11 | 26.43 | | | 591.25 | | 0.01 |
| 12 | 34.07 | | | 573.2393 | | 0.04 |
| 13 | 37.93 | Impurity H | 541.2497 | 541.2499 | 0.35 | 0.12 |
| 14 | 53.51 | | | 416.1655 | | 0.02 |
| 15 | 58.02 | Impurity D | 432.1606 | 432.1607 | 0.32 | 0.02 |
| 15 | 58.02 | | | 362.1187 | | 0.02 |
| 16 | 59.34 | Impurity D | 432.1606 | 432.1605 | -0.15 | 0.07 |

From a comparison of the respective blanks and sample measurement, it is possible to reliably confirm the elution of the target components from the 2nd dimensional column. From the results of the scan analysis by the LCMS-9030, it is possible to estimate the molecular weights of the target impurities by investigating the ions observed specifically at the elution times for the applicable peaks. The measurement results in positive mode and negative mode are summarized in Tables 3 and 4. Additionally, atorvastatin, the main component, and the impurities noted in the European Pharmacopoeia (EU) were attributed with high mass accuracy by comparison to the theoretical mass values.

Table 4 Results of Measurements of the Impurities with the LCMS-9030 (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 |

Further, when an MS/MS scan was used, it was possible to obtain not only the molecular weight information for the impurities, but also a structural analysis from the product ion information. As an example, Impurity F noted in the EP was analyzed with high mass accuracy using MS/MS. As shown in the example in Fig. 3, the cleavage positions were automatically attributed using the MS Workbook Suite software from ACD/Labs.

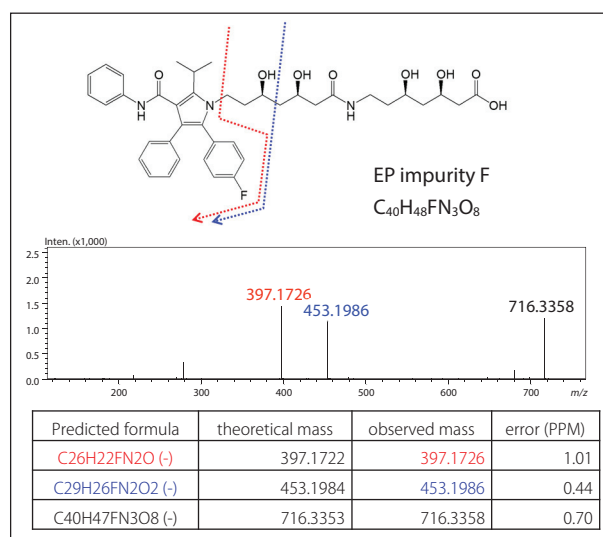


Fig. 3 Results of an MS/MS Analysis of Impurity F noted in the EP (Negative Mode)

Summary

As shown here, it was possible to identify impurity peaks with high probability using nonvolatile 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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