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Application News

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Liquid Chromatography Mass Spectrometry

Analysis of Impurities in Pharmaceutical Ingredients Using Trap-Free Two-Dimensional HPLC and Triple Quadrupole LC/MS/MS (LCMS-8040)

Controlling and confirming trace impurities contained in products in terms of the type, quantity, and safety has become an increasingly important issue to guarantee the product quality in a wide range of fields, including drugs (final formulations and raw materials, generic drugs), foods (health foods, supplements), and fine chemical products (solvents, paints, surfactants, many other synthetic products).
Mass spectrometers, such as triple quadrupole LC/MS/MS instruments, have been attracting attention as a useful means of measuring trace impurities in products. However, its widespread adoption has been complicated by the fact that HPLC-UV methods, which are commonly used for impurity analysis, use non-volatile mobile phase conditions incompatible with LC/MS analysis. To address this problem, laboratories have attempted to modify these methods to make them compatible with LC/MS. However, due to the risk and difficulty associated with changing method conditions, including changing the order of elution and missing impurities that elute near the principle component, very careful consideration is required.
In this report, we introduce an example of analysis in which trap-free two-dimensional HPLC was used to detect impurities using non-volatile mobile phase conditions, which were then converted without complication to volatile mobile phase conditions online to complete the analysis using the LCMS-8040 triple quadrupole mass spectrometer.

HPLC Analysis Using Non-Volatile Mobile Phase (1st Dimension)

A 1 mg/mL rabeprazole sodium test solution was prepared using commercially available laboratory reagents. The sample was then analyzed according to the method described in the Japanese Pharmacopoeia, shown in Table 1. Since phosphate buffer solution is used as the mobile phase, it cannot be introduced directly into the LC/MS.

Table 1 Analytical Conditions

Column	: Shim-pack VP-ODS (150 mm L. × 4.6 mm I.D., 4.6 µm)
Mobile Phase	: Methanol / 50 mmol/L Phosphate Buffer pH 7.0 (3/2)
Flowrate	: 1.0 mL/min.
Column Temp.	: 30 °C
Injection Volume	: 20 µL
Detection	: UV 290 nm

Analysis was conducted using an instrument configuration consisting of a combination of a trap-free two-dimensional HPLC and an LC/MS/MS, as shown in Fig. 1. The mobile phase flow direction differs depending on the valve position associated with each operation. Referring to Fig. 1, the non-volatile mobile phase flow line is indicated in red, the volatile mobile phase flow line in blue, and the impurity fraction peak capture loop in green.

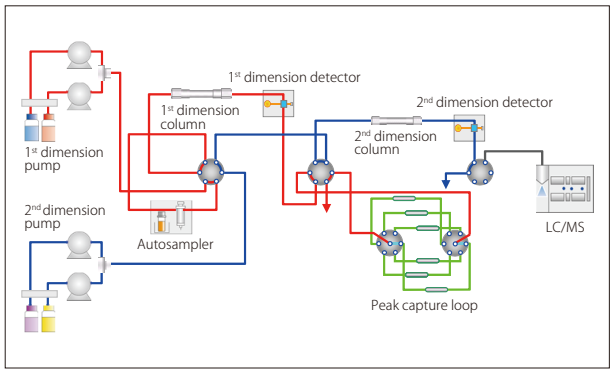

Fig. 1 Flow Diagram

This system was used to analyze a 1 mg/mL rabeprazole sodium solution. The obtained UV chromatogram is shown in Fig. 2. The principle component, rabeprazole, eluted at 5.3 minutes, and several impurity peaks are noticeable in that vicinity before and after that peak. Of these, the four impurity substances shown in the figure were fractionated using the peak capture loop.

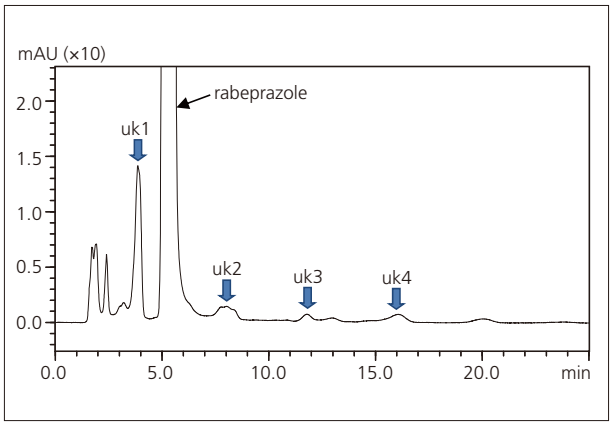

Fig. 2 UV Chromatogram of Rabeprazole Sodium (1st Dimension)

LC/MS Analysis Using MS Compatible Mobile Phase (2nd Dimension)

The impurities (uk1 – uk4) fractionated in the peak capture loop are forced out of the loop by the MS compatible volatile mobile phase due to switching of the valve position and activation of the second dimension pump to introduce each of the peaks into the LC/MS. The conditions used in the second dimension are shown in Table 2.

Table 2 Analytical Conditions (2nd Dimension)

Column	: Shim-pack XR-ODS (50 mm L. × 2.0 mm I.D., 2.2 µm)
Mobile Phase A	: 5 mmol/L Ammonium Acetate – Water
Mobile Phase B	: Methanol
Flowrate	: 0.2 mL/min.
Column Temp.	: 30 °C
Injection Volume	: 20 µL (Loop Volume)
Detection	: UV 290 nm, MS Q3scan (Positive and Negative Mode)

By comparing the UV chromatograms associated with the respective blank measurements and sample measurements, as shown in the LabSolutions LCMS data browser of Fig. 3, it is possible to gain a clear understanding of the elution positions of the target substances from the second dimension column. Further, by examining ions observed in the Q3 analysis results at specific peak elution times, it is possible to deduce the molecular weight of each of the target impurities. In the case of uk-1, as a peak without blank data, *m/z* 376 is observed as positive, and *m/z* 374 is observed as negative, verifying that uk-1 is an impurity with a molecular weight of 375. Table 3 summarizes the results obtained from analysis of the LC/MS data for each impurity.

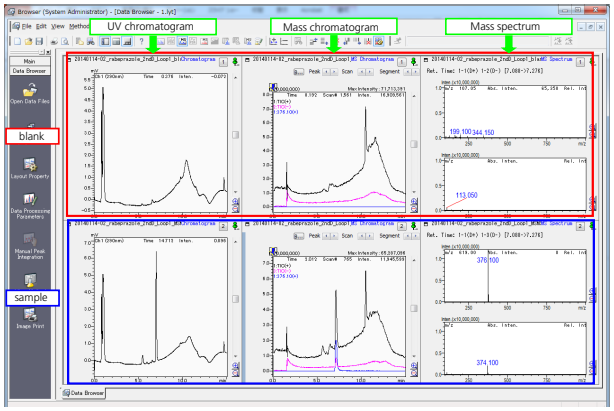

Fig. 3 Analysis Example Using Data Browser (uk-1)

Table 3 Analytical Results of Respective Impurities by LC/MS

compounds	content percentage (%)	*m/z*		deduced MW
		positive	negative	
rabeprazole	99.045	360.20	358.15	359
uk-1	0.433	376.15	374.15	375
uk-2	0.081	394.15	392.10	393
uk-3	0.023	344.20		343
uk-4	0.046	270.20		269

Also, when LC/MS/MS is used, not only can impurity molecular weight information be obtained, but by comparing the fragmentation patterns of the principal component and impurities following the product ion scan, it is possible to predict impurity structures. One example of this is seen in Fig. 4, which shows the product ion scan results for the principal component and uk-1. As the molecular weight of uk-1 was determined from the Q3 scan results to be 375, the mass difference between that and the principal component rabeprazole becomes 16 Da.

Furthermore, by comparing the two product ion scans, it was clear that many of the fragment ions or cleavage positions were the same. From this, it was obvious that uk-1 had a structure similar to that of the principle component. Furthermore, the product ions enclosed in red were specifically observed to have a molecular mass difference of 16 Da. At the position where this ion appears, it is possible to predict the difference in structure. Fig. 4 shows the predicted structure of uk-1.

Thus, by combining trap-free two-dimensional HPLC and LC/MS/MS, it was possible to identify and predict the structure of an impurity peak with high accuracy. At the same time, the non-volatile mobile phase conditions used in the Japanese Pharmacopoeia method could be retained when coupled with LC/MS compatible conditions by the system.

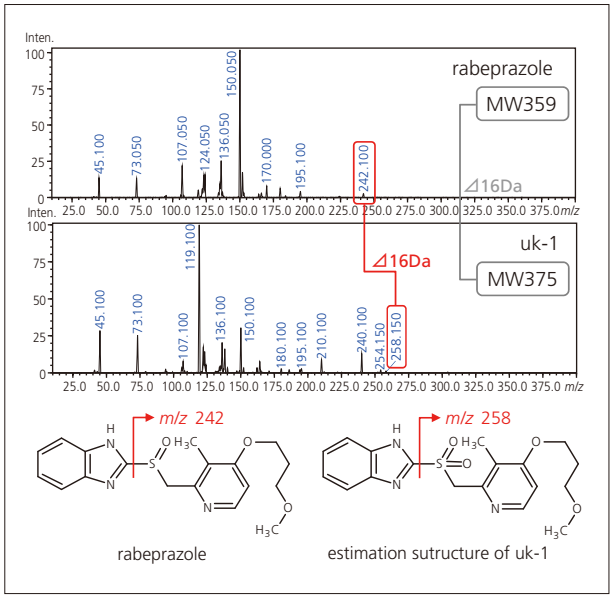

Fig. 4 MS/MS Analysis of Rabeprazole and uk-1

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