Improved Drug Impurity ID Efficiency under CMC using 2-D LC/MS

Technical Report vol. 39

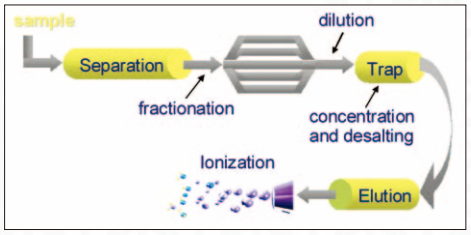
1. Introduction

The analytical method development section in CMC (Chemistry, Manufacturing and Control) department of pharmaceutical company constitutes part of the pharmaceutical developmental process that deals specifically with the physical nature of a drug substance and the drug product, how it is made, and the control of the manufacturing process to provide reliable and reproducible product. HPLC systems in these areas are utilized for the specification tests of active pharmaceutical ingredients (API) to insure the quality of the product as well as the level of impurities.

Traditionally, non-volatile mobile phases containing phosphate buffer solutions were used in the HPLC test methods for QA/QC. When LC/MS analysis is performed, it is necessary to change from a mobile phase containing non-volatile additives to a completely volatile mobile phase that is suitable for atmospheric pressure ionization techniques. This change in analytical conditions may cause changes in the elution order of analytes as well as the possibility of obscuring impurities due to their close proximity to the chromatographic peaks of the major compound. This change in method conditions requires attention to detail and a great deal of effort by the user. Furthermore, recent changes in regulation of impurities along with the globalization of the supply chain have led to even greater demand for impurity identification. Of course, this is causing a bottleneck due to the necessity to modify HPLC conditions that conform to the already established validated test methods, and the need to satisfy demands from the manufacturing section for identification of impurities. Against this backdrop, it is no wonder that a new analytical system is eagerly anticipated.

2. Development of Co-Sense for LC/MS System

To address the demand for LC/MS analysis which would allow utilization of existing non-volatile mobile phase analytical conditions, Shimadzu developed the Co-Sense for LC/MS system (Co-Sense: Collaboration of Shimadzu and Eisai for New Systematic Efficiency) in collaboration with Eisai Pharmaceuticals in 2000. The concept of this system is illustrated in Fig. 1.



Step1: 1D separation

Step2: Peak Fractionation

Step3: Trapping

Step4: 2D separation

Step5: MS Detection

Fig.1: Overview of Co-Sense for LC/MS System

Sample constituents separated using the non-volatile mobile phase are individually diverted from the loop and then transported to the trap column along with a diluent for concentration and desalting. The components that are temporarily retained in the trap column are eluted in the next step, and after separation in the analytical column, are introduced into the MS. This system configuration addresses the requirements of LC/MS analysis without having to eliminate the use of the non-volatile based mobile phase analytical conditions for the primary separation. However, the drawback of this approach is the need for a trap column, which necessitates optimization of the conditions to retain impurities whenever a new compound of interest is to be analyzed. This trapping stage also adds to the overall time required for analysis. Since concentration is not needed in many cases by high sensitivity MS, a smaller volume loop can be used, which reduces the influence of salts on the MS analysis. The advantage of simplifying the flow path is that the time and effort required for optimizing the conditions can be shortened. This analysis of the system overall led to the construction of the current Co-Sense for LC/MS system with a modified flow path.

3. Trap-Free 2D-LC/MS Impurity Identification System

A photograph of this system is shown in Fig. 2, and the flow diagram is shown in Fig. 3. As shown in Fig. 3, the trap column has been removed from the Co-Sense system, and a UV detector has been added to the second-dimension HPLC flow path. Desalting is executed using a divert valve installed just before the MS inlet. The addition of the UV detector to the second dimension greatly facilitates the detection of impurities. In an actual sample workflow, both impurity data and the corresponding blank data are acquired, and the impurity peaks are identified through comparison of the respective chromatograms. Peak isolation and fractionation is conducted using multiple valves in specific combinations. Creating the peak isolation program is easy using the provided macro program. The macro automatically specifies the best valve sequence, requiring only that the user enter the retention times of the first dimension separation. To make operations even easier, a separate macro-program, which automates the construction of a batch schedule for acquiring multiple impurity and blank data, is also provided.

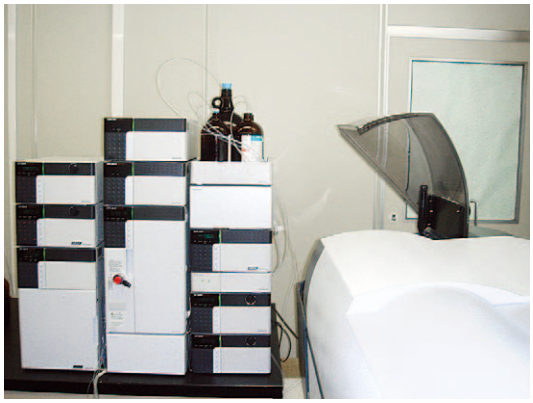


Fig. 2: Photograph of 2D-LC/MS Impurity ID System

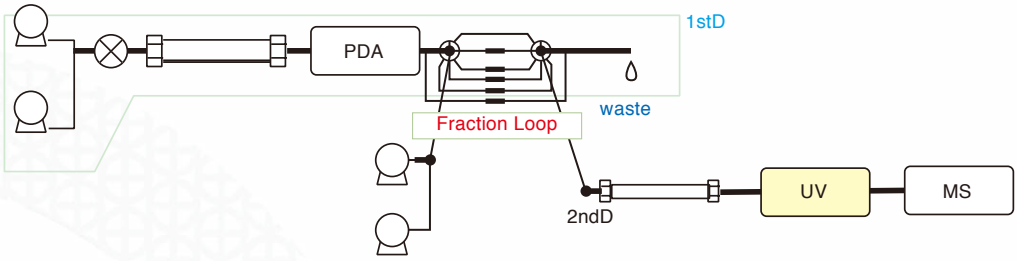


Fig. 3: Basic Flow Diagram of Trap-Free 2D-LC/MS Impurity ID System

4. System Overview

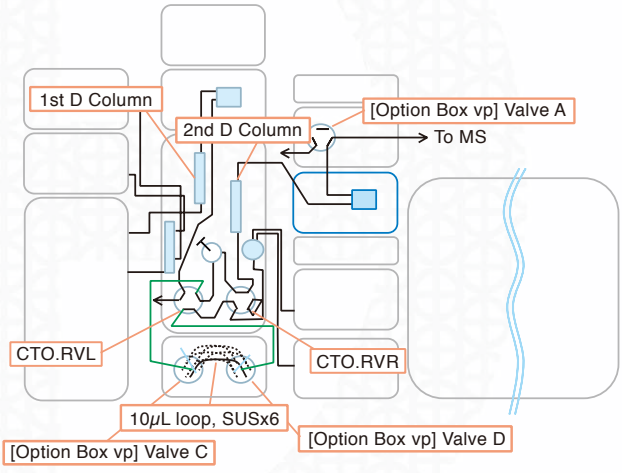


Fig. 4: System Flow Diagram

A flow diagram of this system is shown in Fig. 4, and a photograph and flow diagram of the fractionation loop valve unit are shown in Fig. 5 and Fig. 6, respectively. Since the injection volume for the second-dimension LC is not specified, the sixth loop is never used. The loop sizes are available in 5 μL, 10 μL, 20 μL, and 50 μL capacities.

Fig. 5: Fractionation Loop Valve Unit

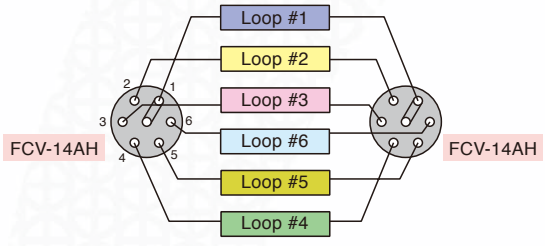
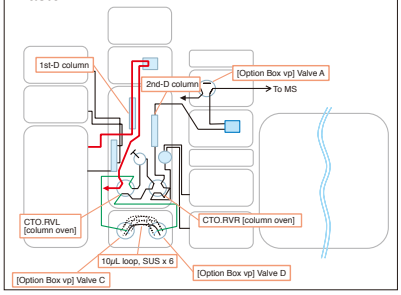


Fig. 6: Flow Diagram of Fractionation Loop Valve Unit

5. Valve Sequences

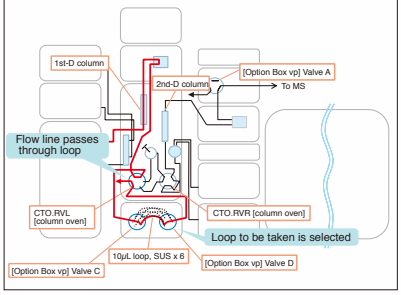
Initial State

After passing through the detector, the non-volatile based mobile phase flows directly to waste.



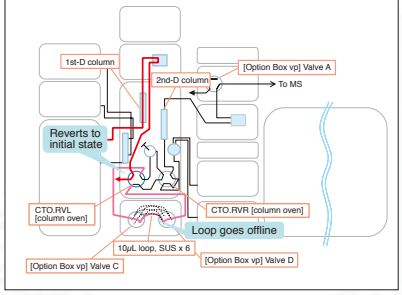
Just Before Impurity Peak Elution

The non-volatile based mobile phase flows to the waste via the specified loop.



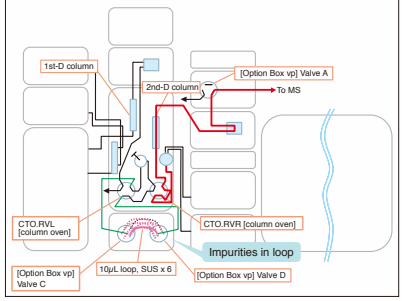
When Peak Top Arrives at Loop Center

Impurities are collected and remain in the loop.



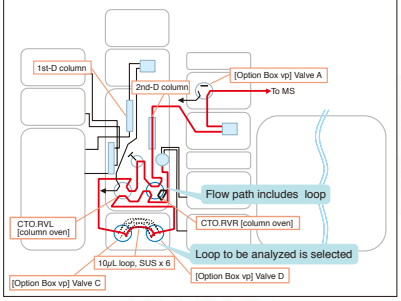
Initial State

The non-volatile based mobile phase flows to the MS (or waste) without passing through loop.



During Analysis

The non-volatile based mobile phase flows to the MS while pushing impurities out of loop.



6. First-Dimension LC Time Program

Figures 7–9 show an overview of the macro tool used for creating the impurity fractionation time program. First, the retention times of the impurities to be fractionated are entered. Up to five retention times separated by commas can be entered at once. If there are six or more impurity peaks, the same entry procedure is repeated for additional impurities, up to the tenth impurity, and so on. If valves C and D are at position “1”, fractionation begins at loop 1, and if the CTO.RVL position is set to “1”, fractionation is ended. The operation is the same for loop 2 and on.

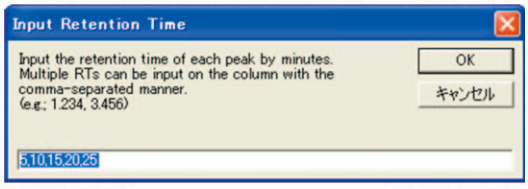


Fig. 7: Impurity Retention Time Entry Window

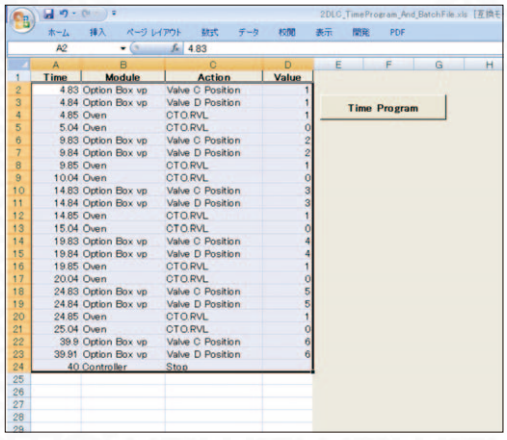


Fig.8: Time Program Created with Excel Macro - To be Pasted into LCMSsolution Time Program.

Time	Unit	Process Command	Number	
0.1	Pump	T.Flow3	0	
3.12	Option Box vp	Valve C Position	1	Loop #1
3.13	Option Box vp	Valve D Position	1	
3.14	Column oven	CTO.RVL	1	End #1
3.64	Column oven	CTO.RVL	0	
4.13	Option Box vp	Valve C Position	2	Loop #2
4.14	Option Box vp	Valve D Position	2	
4.15	Column oven	CTO.RVL	1	End #2
4.65	Column oven	CTO.RVL	0	
6.13	Option Box vp	Valve C Position	3	Loop #3
6.14	Option Box vp	Valve D Position	3	
6.15	Column oven	CTO.RVL	1	End #3
6.65	Column oven	CTO.RVL	0	
17.24	Option Box vp	Valve C Position	4	Loop #4
17.25	Option Box vp	Valve D Position	4	
17.26	Column oven	CTO.RVL	1	End #4
17.76	Column oven	CTO.RVL	0	
19.54	Option Box vp	Valve C Position	5	
19.55	Option Box vp	Valve D Position	5	
19.56	Column oven	CTO.RVL	1	
20	Pump	T.Flow3	0	
20.01	Pump	T.Flow3	0.3	
20.06	Column oven	CTO.RVL	0	
20.54	Option Box vp	Valve C Position	6	
20.55	Option Box vp	Valve D Position	6	
20.56	Column oven	CTO.RVL	1	

Fig. 9: Time Program Excerpt

7. Second-Dimension LC Time Program and Batch Sequence

The second-dimension time program and operation sequence are shown in Fig. 10. This time program is common to all of the impurity fractions. The batch sequence for measurement of actual samples and their corresponding blanks can also be generated at one time using the provided macro program (Fig. 11).

Time	Unit	Process Command	Number
0.01	Column oven	CTO.RVL	1
0.02	Option Box vp	Valve A Position	0
1,5	Option Box vp	Valve A Position	1
10	Pump	B.conc3	50
10,01	Pump	B.conc3	10
19	Column oven	CTO.RVL	0
20	Controller	Stop	

Loop and 2nd D go online (sample injection)

Flow line changes from column to Waste (desalting)

Flow line changes from column to MS

Loop and 2nd D go offline

Fig. 10: 2nd-Dimension Time Program

Analysis	Vial#	Tray Name	Inj. Volume	Sample Name	Sample ID	Method File	Data File	Tuning File	Analysis Type
1	1	1	10			D:¥2DLC¥1stD-LC_fractionate.lcm	20100921_1stD-LC_fractionate.lcd		
2	-1	1	10			D:¥2DLC¥2ndD-LCMS_loop6.lcm	20100921_2ndD-LCMS_loop6.lcd		
3	-1	1	10			D:¥2DLC¥2ndD-LCMS_loop1.lcm	20100921_2ndD-LCMS_loop1.lcd		
4	-1	1	10			D:¥2DLC¥2ndD-LCMS_loop2.lcm	20100921_2ndD-LCMS_loop2.lcd		
5	-1	1	10			D:¥2DLC¥2ndD-LCMS_loop3.lcm	20100921_2ndD-LCMS_loop3.lcd		
6	-1	1	10			D:¥2DLC¥2ndD-LCMS_loop4.lcm	20100921_2ndD-LCMS_loop4.lcd		
7	-1	1	10			D:¥2DLC¥2ndD-LCMS_loop5.lcm	20100921_2ndD-LCMS_loop5.lcd		
8	-1	1	10			D:¥2DLC¥1stD-LC_equilibrate.lcm	20100921_1stD-LC_equilibrate.lcd		
9	2	1	10			D:¥2DLC¥1stD-LC_fractionate.lcm	20100921_1stD-LC_fractionate_blank.lcd		
10	-1	1	10			D:¥2DLC¥2ndD-LCMS_loop6.lcm	20100921_2ndD-LCMS_loop6_blank.lcd		
11	-1	1	10			D:¥2DLC¥2ndD-LCMS_loop1.lcm	20100921_2ndD-LCMS_loop1_blank.lcd		
12	-1	1	10			D:¥2DLC¥2ndD-LCMS_loop2.lcm	20100921_2ndD-LCMS_loop2_blank.lcd		
13	-1	1	10			D:¥2DLC¥2ndD-LCMS_loop3.lcm	20100921_2ndD-LCMS_loop3_blank.lcd		
14	-1	1	10			D:¥2DLC¥2ndD-LCMS_loop4.lcm	20100921_2ndD-LCMS_loop4_blank.lcd		
15	-1	1	10			D:¥2DLC¥2ndD-LCMS_loop5.lcm	20100921_2ndD-LCMS_loop5_blank.lcd		

Actual samples

Blank samples

Since these are not to be injected by the autosampler in 2nd D, a "-1" is entered.

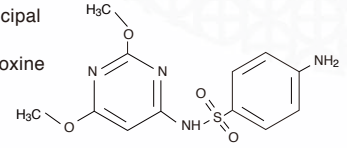
The same set of method files from loop 6 to 1 is repeated twice.

Fig. 11: Batch Sequence Auto-generated with Macro Program is Pasted to LCMSsolution Batch Menu

8. Analysis Example

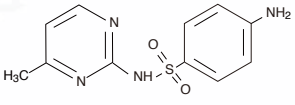
A sample consisting of sulfadimethoxine as the main compound, and four sulfa drugs having a similar structure to serve as impurities is used for this example. The impurities were each mixed with the main compound at percentages of 0.1% each relative to the main compound, which had a concentration of 500 μg/L. The structures of the respective substances are shown below, and the measurement results are shown in Figures 12–16. All of the "impurities" were confirmed in the results.

Pseudo-principal ingredient: Sulfadimethoxine



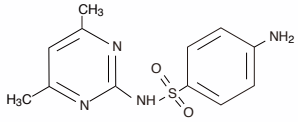
Molecular Formula =$C_{12}H_{14}N_4O_4S$
Monoisotopic Mass =310.073575 Da
$[M+H]^+$ =311.080851 Da

Sulfamerazine



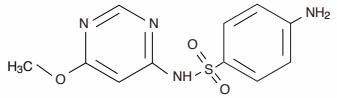
Molecular Formula =$C_{11}H_{12}N_4O_2S$
Monoisotopic Mass =264.068096 Da
$[M+H]^+$ =265.075372 Da

Sulfadimidine



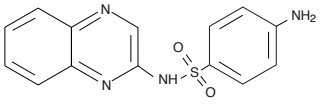
Molecular Formula =$C_{12}H_{14}N_4O_2S$
Monoisotopic Mass =278.083746 Da
$[M+H]^+$ =279.091022 Da

Sulfamonomethoxine



Molecular Formula =$C_{11}H_{12}N_4O_3S$
Monoisotopic Mass =280.06301 Da
$[M+H]^+$ =281.070287 Da

Sulfaquinoxaline



Molecular Formula =$C_{14}H_{12}N_4O_2S$
Monoisotopic Mass =300.068096 Da
$[M+H]^+$ =301.075372 Da

Analytical Conditions < LC 1st Dimension >

Column:	Shim-pack VP-ODS, 150mm L. × 4.6mm i.d., 5μm
Mobile phase:	0.01mol/L phosphate buffer solution (pH 2.6) methanol mixture (7:3)
Mobile phase flow rate:	1 mL/min
Column temperature:	40 °C
Sample injection volume:	10 μL
PDA detection wavelength:	200–350 nm (270 nm monitored)

Analytical Conditions < LC 2nd Dimension >

Column:	Shim-pack XR-ODS 75mm L. × 2.0mm i.d., 2.2 μm
Mobile phase A:	0.1% formic acid aqueous solution
Mobile phase B:	Methanol
Mobile phase ratio:	10%B(0min)-50%B(10min)-10%B(10.01–20min)
Mobile phase flow rate:	0.3 mL/min
Column temperature:	40 °C
Sample injection volume:	10 μL (loop volume)
UV detection wavelength:	270 nm

<MS>

Ionization mode:	ESI^+
Nebulizer gas flow rate:	1.5 L/min
Drying gas pressure:	0.15 MPa
Impressed voltage:	4.5 kV
CDL temperature:	200 °C
BH temperature:	200 °C
Scan range:	*m/z* 100–1000

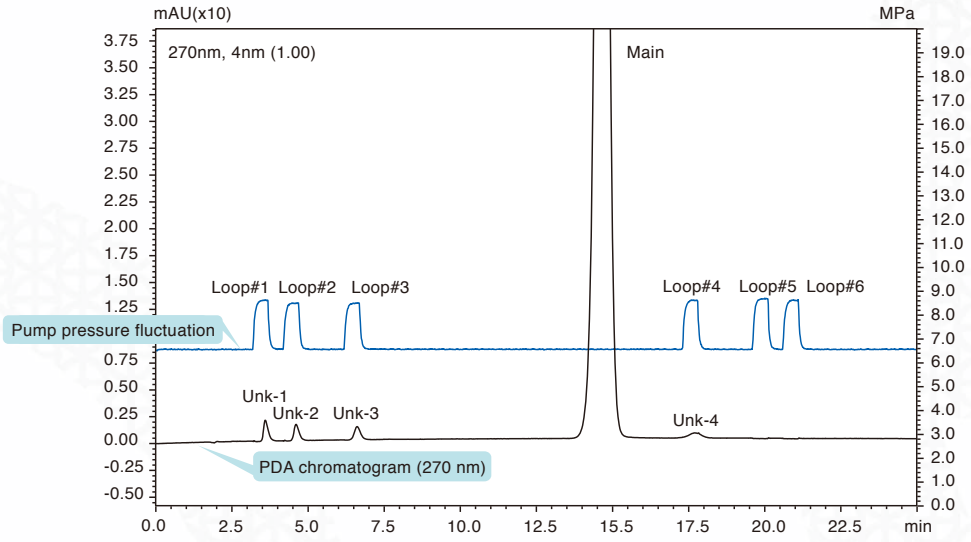


Fig.12: 1st-Dimension PDA Chromatogram
The blue-colored trace displays the system pressure, and the black-colored trace shows the UV 270 nm signal. These correlate perfectly, indicating that the elution peaks are reliably fractionated in the loop.

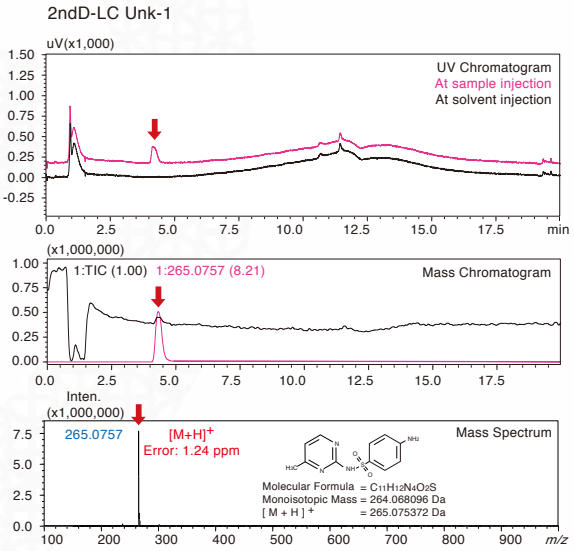


Fig. 13: UV Chromatogram, MS Chromatogram and MS Spectrum of Unk-1
The pink trace in the UV chromatogram refers to when the sample was injected, the black trace is for the solvent injection (blank), and the red arrow indicates the impurity.

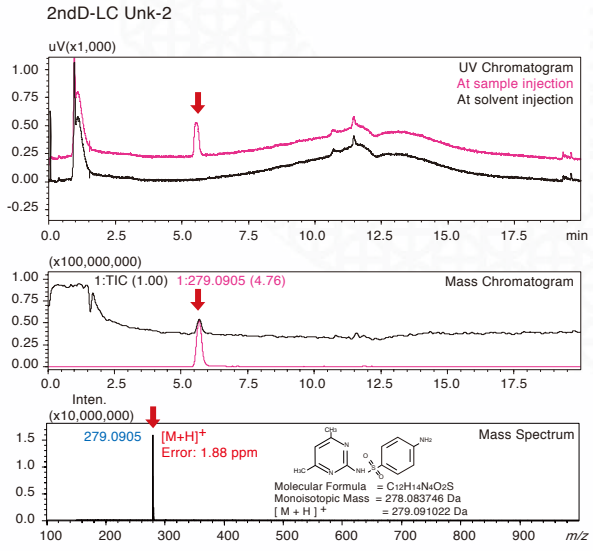


Fig. 14: UV Chromatogram, MS Chromatogram and MS Spectrum of Unk-2

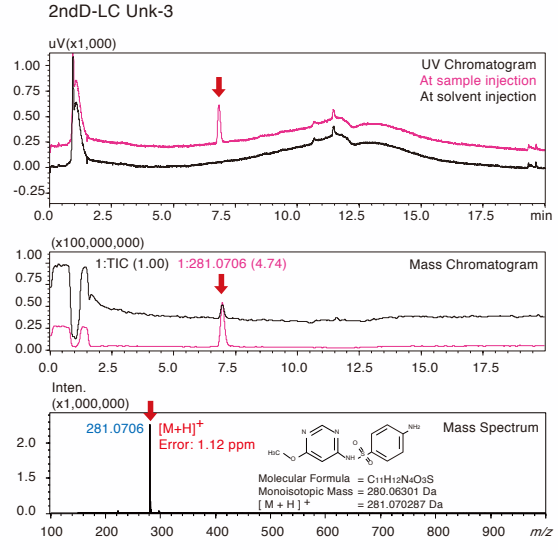


Fig. 15: UV Chromatogram, MS Chromatogram and MS Spectrum of Unk-3

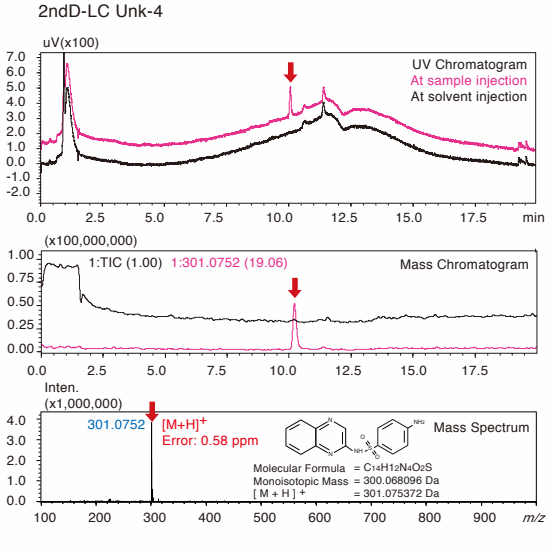


Fig. 16: UV Chromatogram, MS Chromatogram and MS Spectrum of Unk-4

9. Utilizing the Data Browser

The Data Browser provided in LCMSsolution offers additional functionality that enhances the efficiency of impurity analysis-related tasks. The layout template for displaying data can be customized and saved beforehand. As shown in Fig. 17, the stored layout was constructed so that the upper tier is reserved for display of the UV chromatogram and MS spectrum of impurity-related data (2-D LC), and the lower tier for display of the UV chromatogram and MS spectrum of the blank data corresponding to the impurity peak (2-D LC). In this case, the Unk-1 (sulfamerazine) data and the respective blank data are displayed using the “drag and drop” function from the Data Explorer for display in the Data Browser. The differences in retention times between those obtained with the UV detector and those with the MS were synchronized using the retention time correction function provided in the browser. The retention time correction function is stored in the method file, and it can be set up beforehand, so it needs not be set up each time. Furthermore, utilization of this function permits automatic detection of the impurity peak from the UV chromatogram, and the corresponding display of the MS spectrum. In addition, since all of the data files are processed automatically, identification of the impurity on the UV chromatogram not only allows synchronous display of the corresponding impurity spectrum, but also synchronous display of the blank MS spectrum, as well. The actual operation is conducted by just using the mouse to drag from the start of the impurity peak in the impurity UV chromatogram to the end of the peak. Upon releasing the mouse button, the averaged corresponding impurity spectrum and blank spectrum will be displayed.

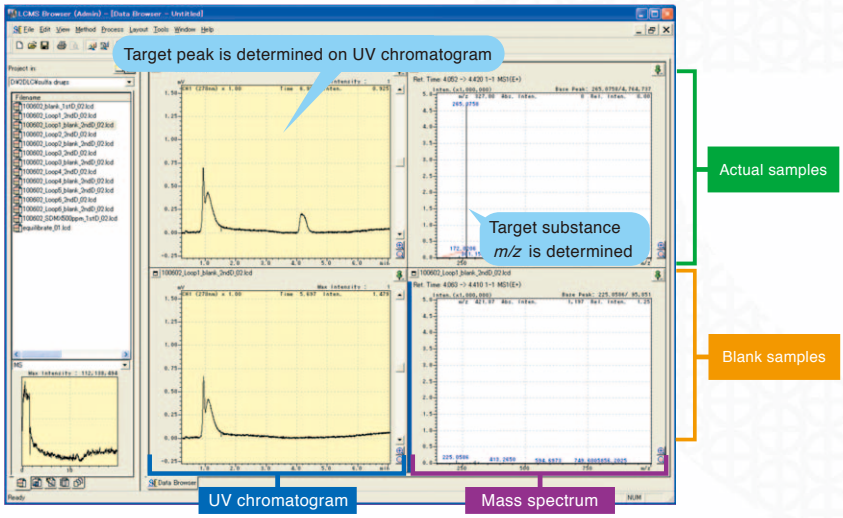


Fig. 17: Unk-1 and Blank Data Displayed in Data Browser

10. Summary

When using the two dimensional LC system introduced here, retention time repeatability in the first dimension is extremely important because impurity peak fractionation is conducted within specific time-controlled intervals. In other words, this technique cannot be applied unless retention times are consistent. However, it is reasonable to assume that the repeatability of retention times has already been determined since retention time repeatability is fundamental to the development of a specific test method. If the separation technique for the first dimension separation is already established, measurement can be conducted without re-evaluation of the technique introduced here, eliminating the need for LC/MS measurement using complex volatility conditions that would have been required otherwise. The ability to confirm impurities through comparison with blank data should greatly contribute to the efficiency of CMC impurity identification.

Printed in Japan, September 2011

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Printed in Japan 3295-08105-10ANS