SHIMADZU

LC-MS Application Data Sheet No. 038

Structure elucidation of trace amount of impurities in drugs using Co-Sense for LC-MS system

Checking the molecular structure of impurities and decomposed substances is an important step to ensure quality in the drug development. In addition, identification of structure of impurities comprising more than 0.1% of a drug has become an obligation with the implementation of the ICH guidelines. HPLC, and particularly LC-MS, is an indispensable tool of analysis for this purpose. One problem that arises in this case is that, although many of the HPLC applications require buffer solutions for optimal separation, the LC and MS interface does not tolerate non-volatile buffer solutions. In order to solve this problem, a step is necessary where the LC fraction substance is desalinated before MS measurement. This solvent replacement procedure is extremely difficult to perform offline. However, employment of column switching technique allows this to be automatically processed online, as well as preliminary concentration of the target component at the same time.

The Co-Sense for LC-MS system uses the column switching technique to perform online solid-phase extraction, and automates the pretreatment of samples, which is indispensable to LCMS analysis. Complex pretreatment processes such as concentration, desalination, and re-separation are accomplished by exercising simple control through the dedicated software. With this system, the optimal mobile phases for LC separation and MS detection can be chosen independently.

Fig.1 shows the flow path diagram of the Co-Sense for LC-MS system. This system is comprised of 3 LC sections, which are linked using the switching valves (V1, V5). The first LC is for separating the target component under optimal conditions, and non-volatile mobile phases such as a phosphate buffer can be used. The eluted target component is fractionated in the sample loop. This is configured for multiple components by switching the 6-way valve (V2, V3). In the second LC, the target component is trapped in the column (C2) after the fraction solution is automatically diluted with water or other liquid. The buffer solution is removed by cleaning the trapping column. Subsequently, the target component is led to the third LC and is separated again with a mobile phase that is optimized for the LC-MS, and is then introduced into the mass spectrometer. Mass spectrometry is performed using the single stage quadrupole Shimadzu mass spectrometer capable of ESI and APCI ionization methods.

Shown here is an example of the analysis of impurities comprising about 0.1% of the principal agent by UV area ratio.

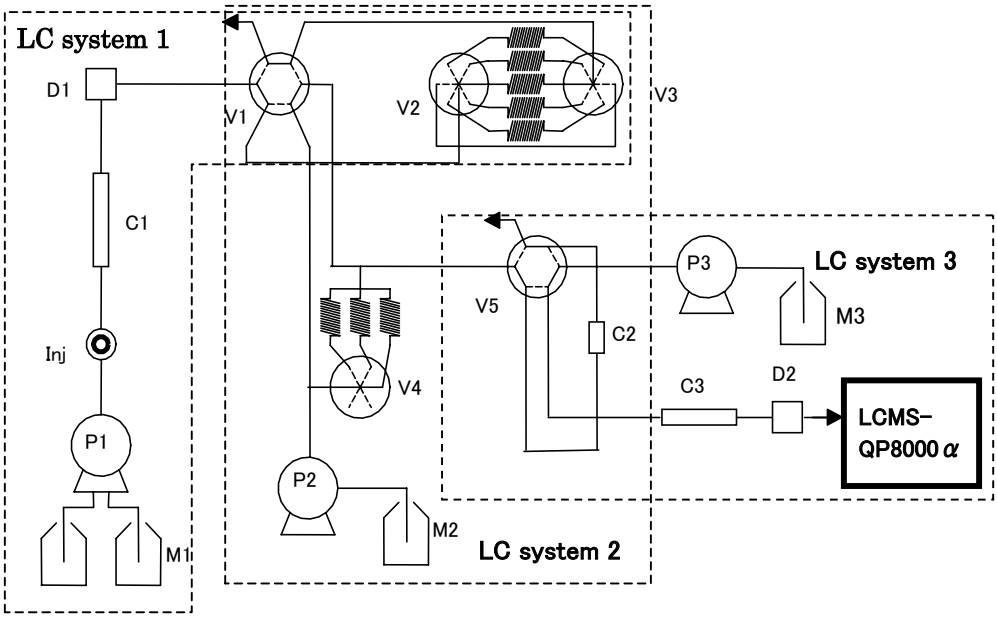
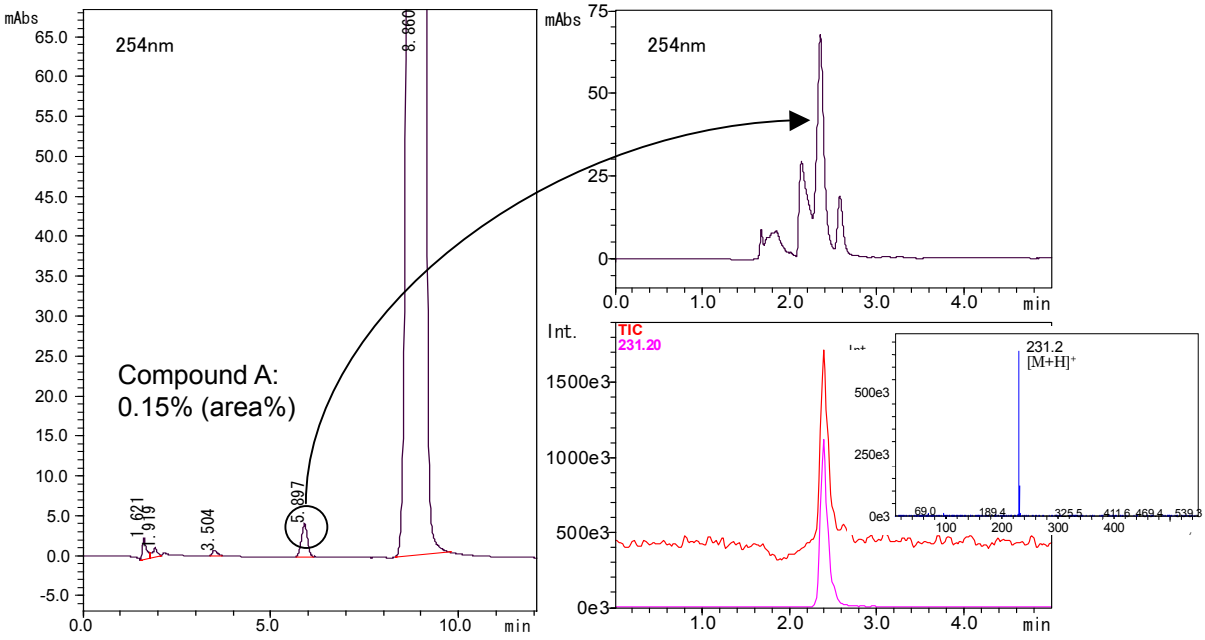


Fig. 1 A schematic diagram of the tandem HPLC system with mass spectrometer

This system was applied to an actual analysis of trace constituents in drugs, and Fig.2 shows the data of the verified MS spectrum. In the preparative chromatogram of Fig.2a, the main component has been eluted at 8.9min, and an impurity peak of UV area ratio 0.15% (compound A) has been detected at 5.9min. This compound A has been measured by MS in the APCI positive mode after several stages of automatic pretreatment, including (1) fractionation in the loop, (2) adsorption in the trapping column, (3) cleaning of the phosphate, and (4) driving out from the trapping column by methanol and introduction into the semi-micro column for re-separation. Fig.2b shows the UV chromatogram, total ion chromatogram, and mass spectrum from the third LC. From the verification of the protonated molecule $[M+H]^+$ of m/z 231, it is assumed that compound A is a compound with a mass number of 230.

To summarize, the following can be raised as the characteristics of the Co-Sense for LCMS.

1. Large quantity injection is possible by using a column with a large internal diameter in the first LC. This means that sufficient amount of samples can be introduced into the MS enabling detection of trace amount of impurities.
2. The fractionated component can be further refined and concentrated in the third LC.
3. The Co-Sense system, which performs the whole process from sample preparation to MS analysis online, is useful for the detection of unstable compounds which decompose during sample preparation in conventional methods.



(a) Chromatogram of preparative separation (b) Rechromatography and MS analysis of compound A

Fig. 2 LCMS analysis of impurities above a level of 0.1%.

Table 1 Analytical conditions

Column1	: Shim-pack VP-ODS (4.6mmI.D.x 150mmL.)		
Mobile phase1	: 20mM (sodium) phosphate buffer pH2.6 / methanol (45 : 55)		
Flow rate	: 1.0mL/min	**Column temperature**	: 40 degred C
Column2	: Shim-pack GVP-ODS (2.0mmI.D.x 5.0mmL.)		
Mobile phase2	: water	**Flow rate**	:1.0mL/min
Column temperature	: room temp.	**Dilution factor**	: 1/3 (6min)
Column3	: Shim-pack VP-ODS (2.0mmI.D.x 150mmL.)		
Mobile phase3	: methanol	**Flow rate**	: 0.2mL/min
Column temperature	: 40 degree C		
Probe voltage	: +4.5 kV (APCI positive)	**Probe temperature**	: 400 degree C
CDL temperature	: 230 degree C	**Nebulizing gas flow**	: 2.5 L/min
CDL voltage	: -10 V	**DEFs voltage**	: 50 V
Scan range	: m/z 10-550 (1.0 sec/scan)		

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