SHIMADZU
Excellence in Science

Technical Report

High-Sensitivity Quantitative Analysis of Trace-Level Impurities and Active Ingredients with HPLC

1. High-Sensitivity Quantitative Analysis of Trace-Level Substances

As the trend toward smaller and smaller analyte concentrations progresses, conventional analytical methods are becoming less and less able to address trace-level analyses. For example, not only do product impurities affect product quality, there are increasing instances of product safety being compromised as well. This has prompted increasingly stringent impurity control across a wide range of business enterprises, where concern about quantitative analysis of extremely low levels of impurities is the current reality. On the other hand, when the analyte is the active ingredient itself, it becomes increasingly difficult to establish a quantitative method as the concentrations decrease to minute levels.

While the typical approach to addressing this situation is to increase sensitivity by taking such measures as increasing the sample concentration, there is always the concern that the solubility of the principal ingredient may be adversely affected, or that the analyte might not be sufficiently separated from possibly large numbers of obstructing substances in the sample matrix (*see* Fig. 1). Addressing this situation often necessitates the use of pretreatment procedures such as extraction and concentration, which also adversely affects efficiency due to the extra time and labor involved in conducting complicated pretreatment procedures.

Thus, it becomes necessary to satisfy two very different requirements for the quantitation of trace-levels of analytes: increasing measurement sensitivity and speeding up operations. The key point in achieving this is to automate the entire process from the fraction collection (fractionation) and concentration of the analytes to final quantitation (*see* Fig. 2)

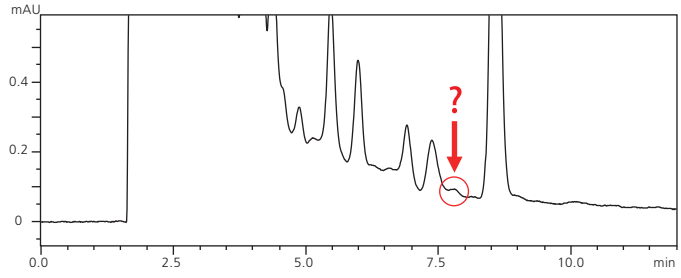


Fig. 1 Example of Impurity Peak

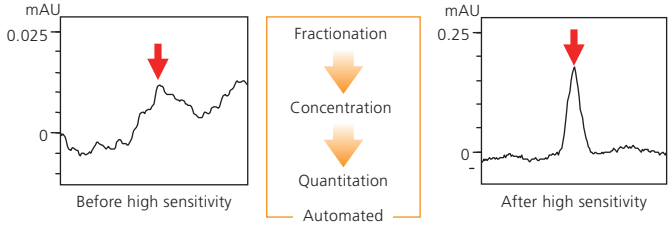


Fig. 2 Automated Quantitative Analysis of Impurities at Micro Levels

2. Automating the Quantitation of Trace-Level Substances

If the functionalities built into the conventional HPLC or UHPLC (ultra high performance HPLC) could be extended to achieve automation of the 3 steps of Fig. 2 ((1) fractionation, (2) concentration, (3) quantitation), it would be of great benefit in terms of reducing costs, and the time associated with operation and maintenance.

This is precisely what is achieved with the unique Co-Sense for Impurities system(Note 1), which can easily be constructed using the inherent flexibility of the HPLC or UHPLC.

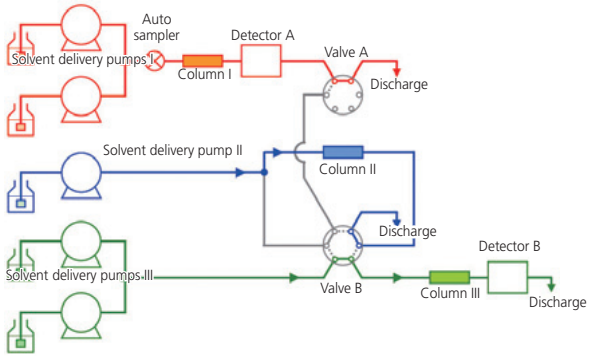


Fig. 3 Flow Line Diagram for Co-Sense for Impurities System

3. Co-Sense for Impurities System

By merging column switching technology and 2-dimensional separation technology, the Co-Sense for Impurities system achieves automation of the processes included in the 3 operational steps, which include fractionation, concentration and quantitation. This system can be constructed not only with Prominence hardware, but with UFLC(XR) and Nexera components as well. Whichever is used to construct the system, quantitation of trace-level substances at high sensitivity and automated operation are both achieved. Here we introduce the principle and some applications of this system.

<<Overall System Flow Line>>

Fig. 3 shows the flow line diagram of the Co-Sense for Impurities system. This system consists of the following 3 flow lines.

(1) Fractionation flow line (red-colored flow line)

(2) Concentration flow line (blue-colored flow line)

(3) Quantitation flow line (green-colored flow line)

<<Step (1): Fractionation>>

The autosampler injects the sample and separation begins on column I. Until the target constituent is completely eluted from the column, the valve A flow line, colored red, is directed as shown in Fig. 3, so that all peaks other than that of the target substance are discharged with the mobile phase to the waste bottle.

Once the target substance has eluted from the column and has passed through detector A, the valve A flow line is switched. This allows the target substance to proceed toward fractionation (colored yellow). The valve A switching start and end times are calculated from analysis results obtained in prior trials.

<<Step (2): Concentration>>

The target substance and any neighboring substances that were sent in the direction of fractionation proceed toward column II after being routed through valve B (*see* Fig. 5). Prior to reaching column II (at the point indicated as "T"), a large volume of new mobile phase is introduced into the flow line from solvent delivery pump II. This new mobile phase is specifically selected to enhance retention of the target substance on column II. For example, in the case of reversed phase analysis, a water-rich composition would effectively provide for concentration and trapping of the target substance (and neighboring substances) on column II.

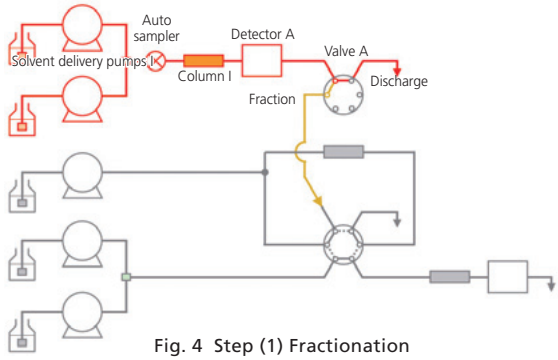


Fig. 4 Step (1) Fractionation

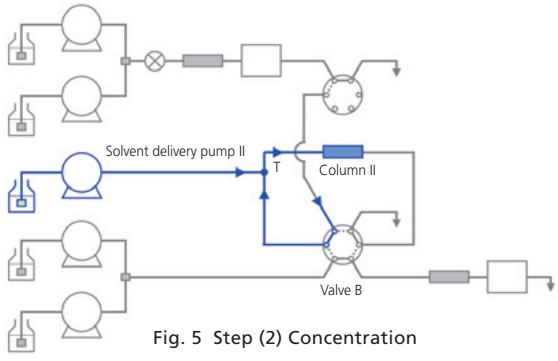


Fig. 5 Step (2) Concentration

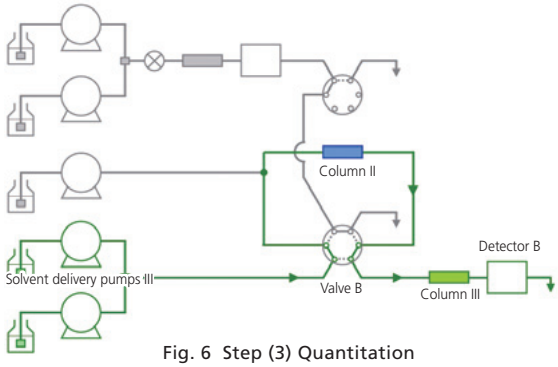


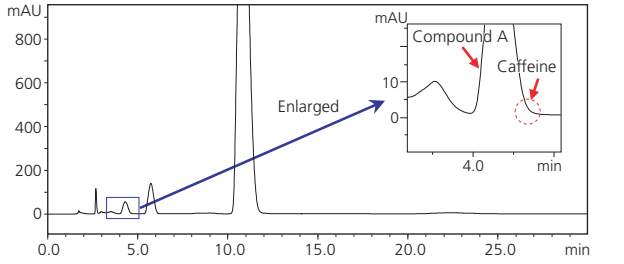
Fig. 6 Step (3) Quantitation

<<Step (3): Quantitation>>

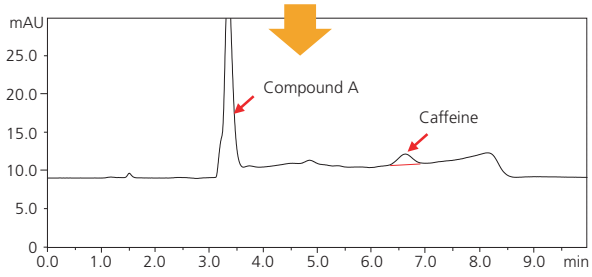
When trapping of the target substance is completed, solvent delivery from pump II ceases, and at the same time, the solvent delivery is re-routed by the switching of valve B as indicated with the green-colored flow line shown in Fig. 6. Since solvent delivery through column II is switched from pumps I and II to that from pumps III, the target substance (and neighboring substances) is eluted from column II and then routed to column III.

Finally, after the target and neighboring substances are further separated with their passage through column III, the target substance is detected by detector B.

As described above, the 2 separation processes (1) and (3), and the concentration process (2) provided with this system allow good separation to be obtained for improved sensitivity, even if a larger sample injection volume is used.



Analytical results by conventional method (chromatogram using detector A)



Analytical results using Co-Sense for Impurities System

<<Step (1) – Fractionation Conditions>>

Column	: Shim-pack VP-ODS (150 mmL. × 10.0 mmI.D., 5 µm)
Mobile phase	: A; 20 mmol/L phosphate buffer solution (pH 2.5)
	: B; Acetonitrile
	Isocratic analysis B.CONC 15%
Flow rate	: 4.7 mL/min
Column temperature	: 40°C
Injection volume	: 1.5 mL
Wavelength	: 272 nm
Fraction interval	: Target peak region (4.21 – 4.86 min)

<<Step (2) – Concentration Conditions>>

Column	: Shim-pack GPRC-ODS (15 mmL. × 8.0 mmI.D., 5 µm)
Additional mobile phase	: 100 mmol/L ammonium acetate aqueous solution
Flow rate	: 12.0 mL/min
Column temperature	: 40°C

<<Step (3) – Quantitation Conditions>>

Column	: Synergi Hydro-RP (100 mmL. × 3.0 mmI.D., 2.5 µm)(Note 2)
Mobile phase	: A; 100 mmol/L ammonium acetate aqueous solution
	: B; Methanol
	Gradient analysis
	B.CONC 20% (0 min)→65% (5 min)→20% (5.01 – 10 min)
Flow rate	: 0.4 mL/min
Column temperature	: 40°C
Wavelength	: 272 nm

Fig. 7 Co-Sense for Impurities System Application Example (1)

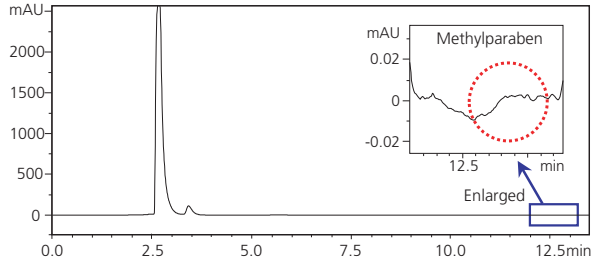
4. Co-Sense for Impurities System Application Example

Fig. 7 and 8 show application examples of trace-level substance analysis using the Co-Sense for Impurities system.

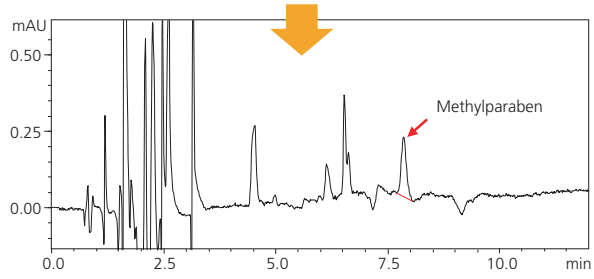
Fig. 7 shows an example of analysis of a drug product (cefazolin 0.5 mg/mL) spiked with the pseudo-impurity, caffeine (equivalent to 0.0008 % of impurity). Here, we used a high-speed analytical column for step (3) in order to speed up the analysis. Fig. 8, on the other hand, shows an example of analysis of a drug product (ritodrine injection solution 10 mg/mL) spiked with the pseudo-impurity methylparaben (equivalent to 0.00001 % of impurity).

In analyses of both Fig. 7 and 8, high separation was achieved using different compositions of mobile phase for each of the 2 separation modes (Step (1) and (3)), in addition to columns having different separation characteristics.

The effect of these measures was to allow the quantitation of trace-level substances, which has been quite problematic using conventional methods.



Analytical results by conventional method (chromatogram using detector A)



Analytical results using Co-Sense for Impurities System

<<Step (1) – Fractionation Conditions>>

Column	: Shim-pack VP-ODS (150 mmL. × 10.0 mmI.D., 5 µm)
Mobile phase	: A; 20 mmol/L phosphate (sodium) buffer solution (pH 2.5)
	: B; Methanol
	Isocratic analysis B.CONC 35%
Flow rate	: 4.0 mL/min
Column temperature	: 40°C
Injection volume	: 200 µL
Wavelength	: 254 nm
Fraction interval	: Target peak region (12.0 – 13.2 min)

<<Step (2) – Concentration Conditions>>

Column	: Shim-pack GPRC-ODS (15 mmL. × 8.0 mmI.D., 5 µm)
Additional mobile phase	: 50 mmol/L phosphate (ammonium) buffer solution (pH 6.8)
Flow rate	: 8.0 mL/min
Column temperature	: 40°C

<<Step (3) – Quantitation Conditions>>

Column	: Shim-pack VP-ODS (150 mmL. × 4.6 mmI.D., 5 µm)
Mobile phase	: A; 20 mmol/L phosphate (sodium) buffer solution (pH 2.5)
	: B; Acetonitrile
	Gradient analysis
	B.CONC 10% (0 min)→55% (7 min)
Flow rate	: 1.0 mL/min
Column temperature	: 40°C
Wavelength	: 254 nm

Fig. 8 Co-Sense for Impurities System Application Example (2)

5. Co-Sense for Impurities System Operation

To achieve comparable ease of operation with HPLC and UHPLC, the software for the Co-Sense for Impurities system is designed using a GUI (graphical user interface). As is clear from Fig. 10, not only are all the necessary settings consolidated on a single window, the operational state of the system is also presented graphically for easy monitoring, allowing anyone to easily conduct trace-level substance analysis.

Fig. 9 Co-Sense for Impurities System

6. Use of a Mass Spectrometer

Another approach to applying the Co-Sense for Impurities system to quantitation of trace-level substances is to use it in conjunction with a mass spectrometer. Here, use of an ultra high performance LC as the front end LC makes it possible to conduct even faster quantitative analysis.

Since the LCMS-8030 triple quadrupole LC/MS/MS and LCMS-2020 single quadrupole LC/MS offer ultra high performance with 15 msec polarity switching and a 15000 u/sec scan speed, adopting the Nexera as the front end LC makes it possible to conduct ultra fast mass spectrometry that fully utilizes the Nexera's ultra high LC performance.

The decision as to whether to select a Co-Sense for Impurities system with an HPLC (or UHPLC) as its base for trace-level substance quantitation, or rather one based on use of an LC/MS/MS (or LC/MS) should be made in consideration of such factors as initial cost, maintenance cost, analysis frequency and required sensitivity.

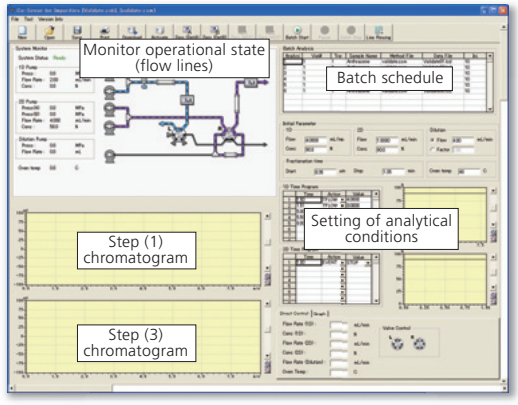


Fig. 10 Co-Sense for Impurities System Operation Window

Fig. 11 Triple Quadrupole LC/MS/MS System
Nexera + LCMS-8030

Fig. 12 Single Quadrupole LC/MS System
Nexera + LCMS-2020

(Note 1) Co-Sense, an acronym for Collaboration of Shimadzu Eisai New Systematic Efficiency, refers to application systems developed collaboratively by Shimadzu Corporation and Eisai Co., Ltd. Presently, automated pretreatment systems for LCMS and NMR (Co-Sense for MS and NMR), a rinse validation support system (Co-Sense for CV) and biological sample analysis system (Co-Sense for BA) are being marketed.
(Note 2) Synergi™ is a trademark or a registered trademark of Phenomenex, Inc. in the United States and other countries.

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