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Application News

High Performance Liquid Chromatograph Nexera™ XR

Simple Labor-Saving Calibration Curve Creation Using Autosampler Automatic Dilution Function

Natsuki Iwata and Ryota Kanamaru

User Benefits

- The autosampler's automatic dilution function eliminates manual dilution preparation, improving operational efficiency and productivity.
- Simply specify the desired dilution ratio in the batch table and use the same method file to automatically dilute solutions and create a calibration curve.
- Setting and management are easy when changing HPLC conditions since a single method file is used regardless of the dilution ratio.

■ Introduction

Dilution of standard and sample solutions for HPLC analysis is generally performed manually using pipettes. However, such work is labor-intensive and time-consuming.

In recent years, automation aimed at labor-saving has become increasingly desirable since if these dilution preparation operations could be automated, work efficiency and productivity would improve.

Nexera Autosamplers are equipped with a pretreatment function that automatically performs dilution, reagent addition, and co-injection. Using this automatic dilution function, it is possible to prepare a sample diluted at a user-defined factor and introduce it directly into the analytical column. This article introduces a simple method for creating calibration curves using the autosampler automatic dilution function.

■ Pretreatment Program and Operation Overview

A method file contains information such as LC parameters, analytical parameters, and the pretreatment program. The pretreatment program can set various dilution ratio, example 100-fold dilution. In addition, when the program is used with the batch add-in (Fig. 2 on the next page), a single method file can be used regardless of the dilution ratio, thereby preventing human errors such as setup mistakes.

The dilution factor and conditions related to the mixing process are configured using the LabSolutions™ workstation. The setup window for the autosampler pretreatment is shown in Fig. 1. Pretreatment program commands are shown in Table 1. In this article, the rinse solution was used as a diluent.

A volume corresponding to the dilution ratio is aspirated from the stock solution vial and dispensed with the diluent into an empty vial (mixing vial) previously set in the autosampler (final volume is 100 µL in this example). The solution in the vial is mixed using the aspiration/dispensing function (pipetting). Finally, a specific amount of the solution is aspirated and injected into the column.

Table 1 Pretreatment Program

Line	Command
1	a3=100/a2
2	n.drain
3	disp 600.0,rs
4	d.rinse
5	vial.n a0,a1
6	n.strk ns
7	aspir a3,ss
8	air.a 0.1,ss
9	d.rinse
10	vial.n rn,sn
11	n.strk ns
12	disp 100.1,rs
13	mix 1,5,40,ss,35
14	n.drain
15	disp 100.0,rs
16	d.rinse
17	inj.p
18	v.inj
19	wait 2.0
20	goto f0
21	end

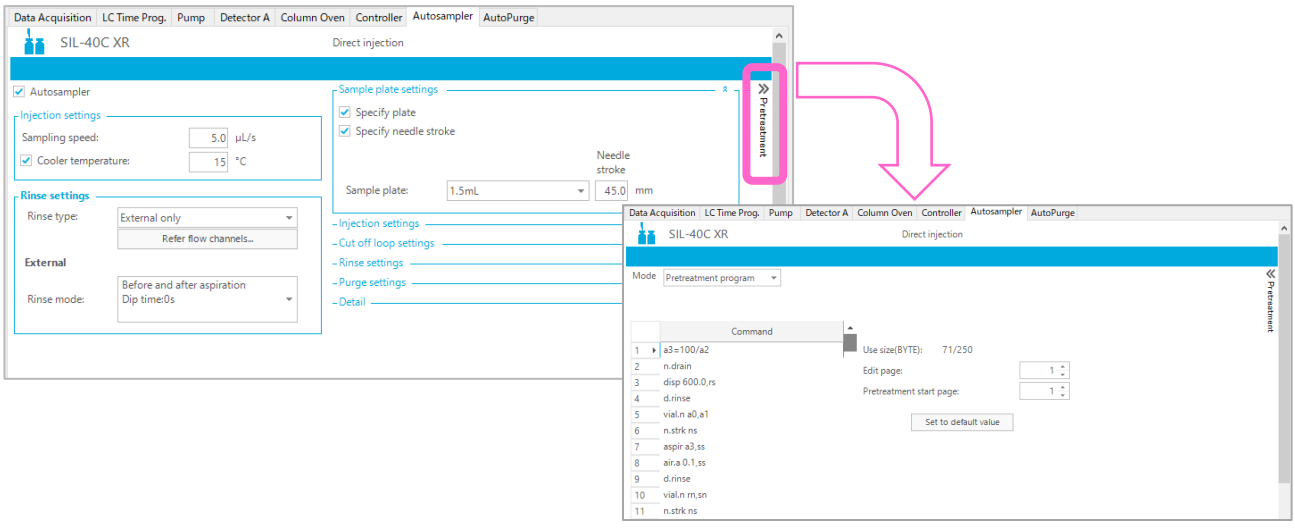

Fig. 1 Setup Window for Autosampler Pretreatment Program

■ Setting up a Batch Table

A batch add-in called the SIL pretreatment variable is pre-applied to LabSolutions to populate the batch table with the location of the stock solution vial and any dilution ratio*1. Fig. 2 shows the SIL pretreatment variable setup window for the batch table. Set the plate number (A0), vial number (A1), and dilution ratio of the stock solution vial (A2) in columns A0-A2 of the SIL pretreatment variables. Place empty vials for automatic dilution (mixing) at the locations specified by the tray and vial numbers in the batch table. If the sample type (standard) and level number are set as shown in Fig. 2, a calibration curve is automatically generated after the analysis. Note that separate analytical parameter settings are required.

*1 Contact Shimadzu for information about applying the batch add-in.

■ Automatic Dilution Analysis for a Caffeine Standard Solution

An automatic dilution analysis was performed using a 250 mg/L caffeine solution. Table 2 shows the analytical conditions. The pretreatment program is the same as in Table 1. 1.0 mL polypropylene vials were used for mixing, and septum vials were used for the stock solutions (standard and sample). Ultrapure water was used as the diluent for the rinse solution*2. Fig. 3 shows the chromatogram of caffeine (concentration after automatic dilution: 2.5 mg/L) diluted 100-fold with ultrapure water.

*2 For HPLC equipped with a multi-rinse function, use the rinse solution specified in the parameter settings of an autosampler.

Table 2 Analytical Conditions

System:	Nexera XR
Column:	Shim-pack™ GIST C18*3 (75 mm × 3.0 mm I.D., 2 µm)
Flowrate:	0.6 mL/min
Mobile Phase:	A) 10 mmol/L (Sodium) phosphate buffer (pH 2.6) B) Methanol
Time Program:	20 % B (0 min)→23 % B (3.30 min)→70 % B (3.31 - 4.30 min) →20 % B (4.31 - 5.50 min)
Column Temp.:	40 °C
Sample:	250 mg/L Caffeine aq.
Injection Volume:	4 µL
Needle Stroke:	45 mm
Vial for Mixing:	Shimadzu Vial, LC, 1 mL, Polypropylene*4
Vial for Stock Solution and Sample:	SHIMADZU LabTotal™ for LC 1.5 mL, Glass*5
Diluent:	Rinse solution (Ultrapure water)
Detection:	272 nm (SPD-M40)

*3 P/N: 227-30002-03, *4 P/N: 228-31600-91, *5 P/N: 227-34001-01

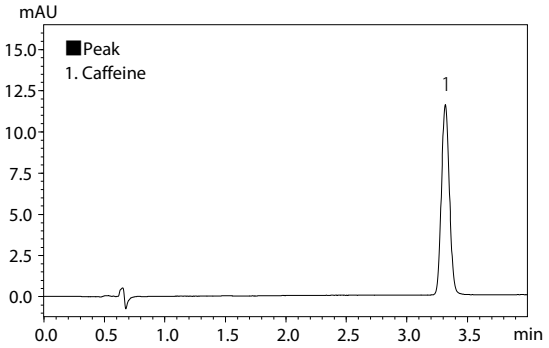

Fig. 3 Chromatogram for the Caffeine Standard Solution
(Dilution Ratio: 100,
Concentration of Caffeine after Automatic Dilution: 2.5 mg/L)

■ Repeatability

A standard solution prepared by automatically diluting 250 mg/L caffeine solution 500-fold with ultrapure water (the concentration after automatic dilution was 0.5 mg/L) was analyzed six times consecutively. The repeatability (%RSD) of the retention time and the peak area are shown in Table 3.

Table 3 Repeatability (%RSD) in Six Replicate Analyses

Retention time	Peak area
0.02	0.96

■ Calibration Curve

The calibration curve for caffeine (concentration range of 0.5-50 mg/L) using the autosampler automatic dilution function was showing an excellent linearity, with a coefficient of determination (r^2) of 0.999 or greater. The calibration curve is displayed in Table 4.

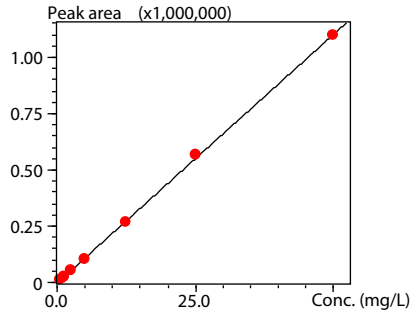

Fig. 4 Calibration Curve

Analysis	Tray Name	Vial#	Sample Name	Sample ID	SIL Pretreatment Variables	Data Comment	Sample Type	Level#	Inj. Volume	Method File
1	1	1	Caffeine	500	A0=1;A1=53;A2=500	A0: Plate No. A1: Vial No. A2: Dilution ratio	1:Standard(I)	1	4	NS_GE3.lcm
2	1	2	Caffeine	200	A0=1;A1=53;A2=200	A0: Plate No. A1: Vial No. A2: Dilution ratio	1:Standard	2	4	NS_GE3.lcm
3	1	3	Caffeine	100	A0=1;A1=53;A2=100	A0: Plate No. A1: Vial No. A2: Dilution ratio	1:Standard	3	4	NS_GE3.lcm
4	1	4	Caffeine	50	A0=1;A1=53;A2=50	A0: Plate No. A1: Vial No. A2: Dilution ratio	1:Standard	4	4	NS_GE3.lcm
5	1	5	Caffeine	20	A0=1;A1=53;A2=20	A0: Plate No. A1: Vial No. A2: Dilution ratio	1:Standard	5	4	NS_GE3.lcm
6	1	6	Caffeine	10	A0=1;A1=53;A2=10	A0: Plate No. A1: Vial No. A2: Dilution ratio	1:Standard	6	4	NS_GE3.lcm
7	1	7	Caffeine	5	A0=1;A1=53;A2=5	A0: Plate No. A1: Vial No. A2: Dilution ratio	1:Standard	7	4	NS_GE3.lcm

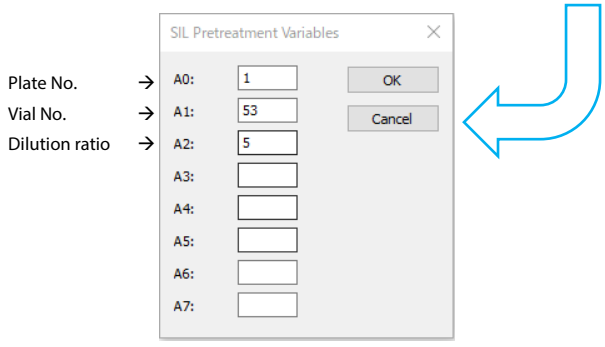

Fig. 2 Setup Window for Batch Table SIL Pretreatment Variables

■ Analysis of Caffeine in Beverages

Commercial green tea and coffee were used as samples. Samples were filtered through 0.2 µm membrane filters.

The pretreatment program and analytical conditions were the same as those in Tables 1 and 2. Figs. 5 and 6 show chromatograms obtained by diluting green tea and coffee 100-fold with ultrapure water using an automatic dilution function. Table 5 shows the analytical results (concentration after automatic dilution).

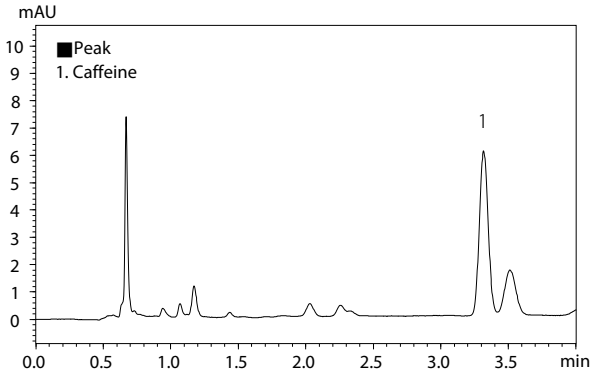

Fig. 5 Chromatogram of Green Tea Obtained by Automatic Dilution (Dilution Ratio: 100)

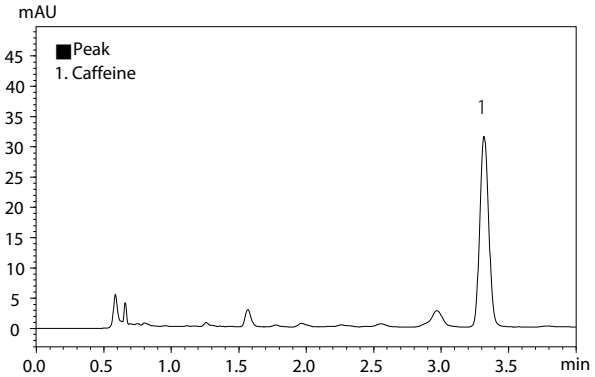

Fig. 6 Chromatogram of Coffee Obtained by Automatic Dilution (Dilution Ratio: 100)

Table 5 Analytical Results (n=6)

Sample	Concentration (mg/L)	%RSD
Green tea	1.32	0.72
Coffee	7.33	1.66

■ Automatic Calculation and Output of Content

The LabSolutions batch option can output directly the "sample concentration" just by specifying the dilution ratio. Fig. 7 shows the setup screen for the dilution ratios and automatic report output in the batch table. Fig. 8 shows the automatically generated report for the green tea analysis.

Sample Name	Dil. Factor	SIL Pretreatment Variables	Report Output	Report Format File
Caffeine	1	A0=1;A1=53;A2=500	☑	Report.lsr
Caffeine	1	A0=1;A1=53;A2=200	☑	Report.lsr
Caffeine	1	A0=1;A1=53;A2=100	☑	Report.lsr
Caffeine	1	A0=1;A1=53;A2=50	☑	Report.lsr
Caffeine	1	A0=1;A1=53;A2=20	☑	Report.lsr
Caffeine	1	A0=1;A1=53;A2=10	☑	Report.lsr
Caffeine	1	A0=1;A1=53;A2=5	☑	Report.lsr
Blank	1	A0=1;A1=54;A2=10	☑	Report.lsr
Coffee	100	A0=1;A1=52;A2=100	☑	Report.lsr
Blank	1	A0=1;A1=54;A2=10	☑	Report.lsr
GreenTea	100	A0=1;A1=51;A2=100	☑	Report.lsr

Fig. 7 Setup Screen for Dilution Ratio and Automatic Report Output in the Batch Table

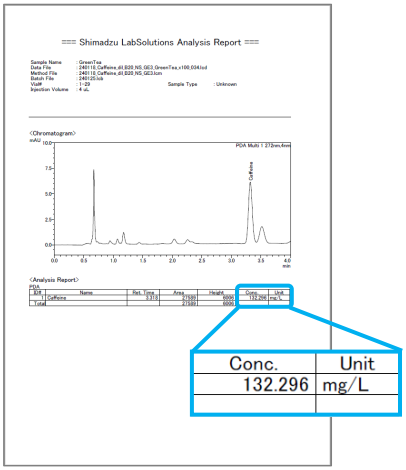

Fig. 8 Automatic Report Output (Green Tea Analysis)

■ Conclusion

By automatically preparing standard solutions for a calibration curve at any dilution ratios and using it for analysis, it was possible to generate an accurate calibration curve. Only stock solutions for standard and sample solutions need to be prepared, and subsequent work, from calibration curve creation to quantification , can be carried out automatically by the HPLC, thereby eliminating the labor needed for pretreatment. The calibration curve method described in this article is effective in improving the operational efficiency and productivity.

Contact Shimadzu for information about applying batch add-ins.

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