Using Autosampler Injector Programming to Determine LOD/LOQ in UHPLC Methods Development

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Abstract

The determination of detection and quantification limits (LOD and LOQ) is essential to good method validation. This poster describes a precise and versatile autosampler (ALS) program method for estimating LOD/LOQ. Typically, an analyte may require many dilution preparations to determine the smallest concentration that can be reliably measured. The ALS is used to systematically dilute analytes to discover the concentration ranges closest to the limit. This has the advantage of streamlining the signal-to-noise ratio and the regression analysis approaches to estimating LOD/LOQ.

Introduction

An approach to establishing the minimum level at which an analyte can be reliably detected is to choose a known concentration and gradually reduce it until the analyte can no longer be quantified or detected. In this experiment, injector programming, a parameter of the OpenLab CDS instrument control method, was employed to automate the sample dilution preparation. A dilution instrument control method was established with injector programming. This method automated the autosampler to deliver precise volumetric amounts into HPLC vials. Using this capability, a stock solution was serially diluted to produce successive samples, each with a ten-fold reduction in concentration.

The analysis method was established based on the HPLC determination of polyphenols using compounds Caffeine and Quercetin. This analysis provided an excellent example for the evaluation of LOD/LOQ.

The versatility of the programming enabled multi-level dilutions with minimal carryover and precision comparable to that obtainable to traditional solution preparation.

Experimental

Systems:
Agilent 1290 Infinity II
High Speed pump G7120A
Multisampler G7167A
Multicolumn Thermostat G7116B
Diode Array Detector G7117A
Control: OpenLAB CDS ChemStation Edition C.01.07 (27)

Column:
Agilent Zorbax SB RRHD, 4.6 × 50 mm 1.8 µm

HPLC:
Mobile phase A: Water (0.1% CH3COOH+ 8 %ACN)
Mobile phase B: Acetonitrile (+ 0.1% CH3COOH)
Dilution flow rate 0.6 mL/min
Analysis flow rate 1.0 mL/min
Injection volume 3 µL
Column temp. 35 °C
Detection 280/4 nm; Reference off
HPLC Gradient Gradient:
0.3 min 10%B
6.0 min 90 %B
Stop Time 5.0 min
Post run time 2.0 min

Figure 1: Agilent 1290 Infinity II

Experimental

The injector program routine is shown in Figure 2. The program is divided into two columns called "function "and "parameter". A core routine with descriptions of each command is shown in Table 1. The routine is repeated for each desired level.

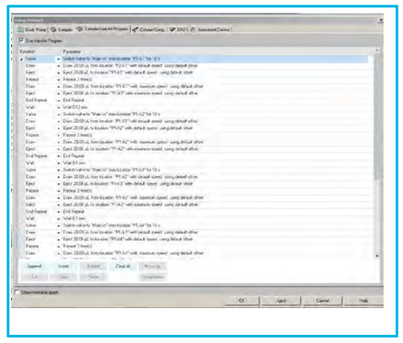

Figure 2: Sample Injector Program

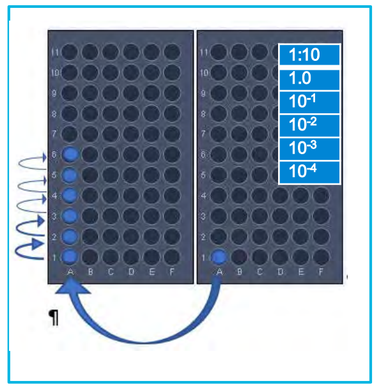

Figure 3: Injector Program Schematic

Table 1. Injector Program Commands

Function	Parameter	Description of Function
Valve	Valve switch to ***Main in*** from location "P1-A1 for 18s	Mobile phase flow is diverted to the specified LC vial location. A precise "diluent" amount is deliver to the vial. e.g. At a flowrate of 0.6 ml/min,180µL is dispensed.
Draw	**Draw** 20µL from location "P2-A1 with default speed using default offset	P2-A1 is the location of the stock solution. The autosampler removes 20 µL.
Eject	**Eject** 20µL from location "P1-A1 with default speed using default offset	. A total of 200µL has been combined for a 10-fold dilution.
Repeat	3 times	All commands bracketed by "Repeat" and "End Repeat" are executed until the parameter is met.
Draw	**Draw** 20µL from location "P1-A1 with maximum speed using default offset	The draw and inject commands in this section provide dynamic mixing.
Inject	**Inject** 20µL from location "P1-A1 with maximum speed using default offset	
End Repeat	**End Repeat**	
Wait	**Wait** 0.1 min	These are the base commands that are repeated for each dilution level.

The 1290 Infinity II autosampler has a maximum injection volume of 20µL in standard configuration. At the dilution flowrate of 0.6 ml/minute, the pump delivers 10µL per second with the flow directed into a 2 mL HPLC vial with insert. The appropriate amount diluent (mobile phase) is delivered and the autosampler injects and mixes the sample for the appropriate dilution ratio.

Results and Discussion

After the dilution method was completed, an analytical method for determination of Caffeine and Quercetin was applied to the series of samples. An example chromatogram is shown in Figure 4.

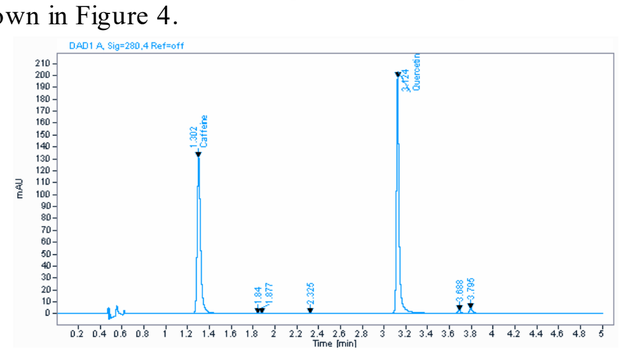

Figure 4: Chromatogram of Caffeine & Quercetin at conc. 40.08 & 102.8 ng/mL

Results and Discussion

Detection limits were visually determined by the analysis of each concentration level and the minimum levels for LOD/LOQ were identified as shown in Figure 5.

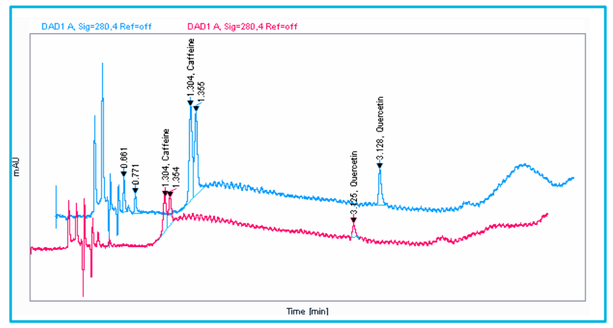

Figure 5: S/N examples 10:1 &3:1

Table 2. Table of Experimental Signal to Noise Ratio Caffeine

Conc. ng/mL	Peak Height	S/N	Area RSD (%)	Area RSD (%)
40.08	126.030	1711	288.262	0.1
4.080	13.168	181	30.043	2.1
4.8×10^{-1}	1.900	54	4.501	9.3
4.8×10^{-2}	0.166	11.3	0.299	12.6
4.8×10^{-3}	0.057	4.0	0.0995	33.7

Table 3. Table of Experimental Signal to Noise Ratio Quercetin

Dilution Level	Peak Height	S/N	Area	Area RSD (%)
10.28	189.833	2578	288.262	0.1
1.028	17.694	244	32.585	0.96
1.028×10^{-1}	2.575	74	4.796	5.3
1.028×10^{-2}	0.458	15.6	0.222	16
1.028×10^{-3}	0.155	5	0.0995	4.4

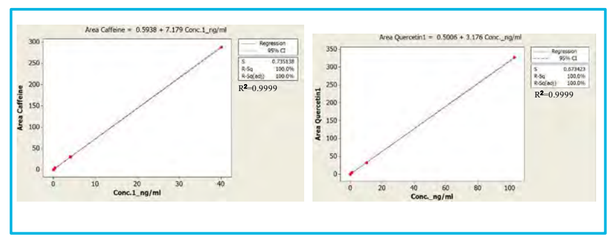

Figure 6: Experimental Regression Analysis for Caffeine and Quercetin

Conclusions

The autosampler injector programming was employed to perform dilutions to estimate LOD/LOQ for Caffeine and Quercetin. The technique streamlined LOD/LOQ determination and reduced sample preparation time. LOD and LOQ limits were determined by visual and signal-to-noise approaches. Although the linear regression curves were excellent, programmed dilutions of 1:1 or 1:5 would provide more points for regression analysis.

References

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(3) Ümit Sengül, Giresun University, Giresun Turkey, 2015
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