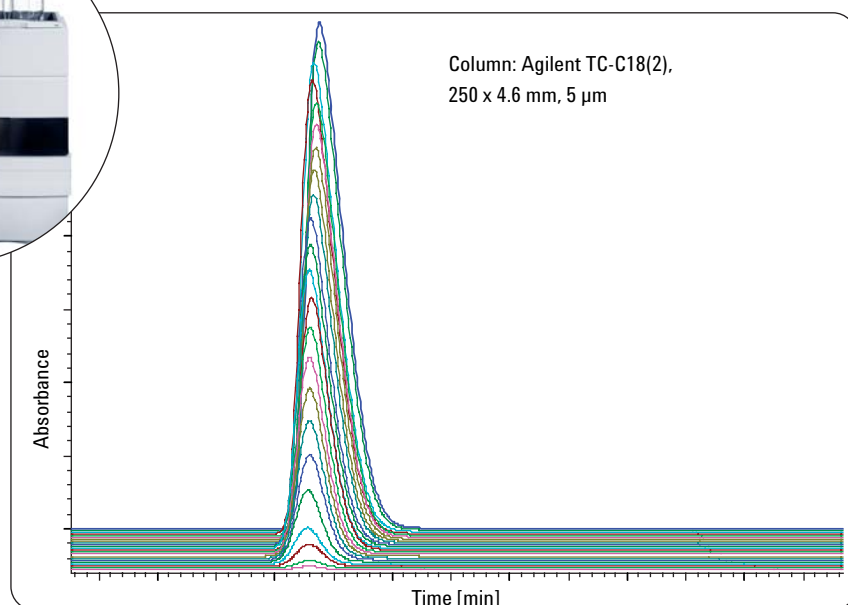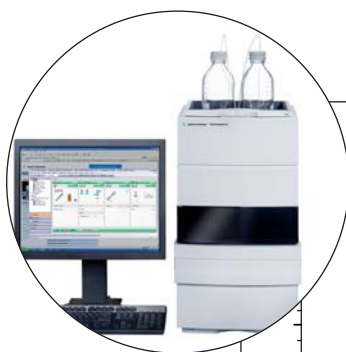# Injector linearity testing for Tramadol quality control with the Agilent 1120 Compact LC and Agilent TC C18(2) columns

Application Note

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Column: Agilent TC-C18(2),
250 x 4.6 mm, 5 µm

Absorbance

Time [min]

Abstract

The Agilent 1120 Compact LC is the system of choice for conventional, analytical-scale liquid chromatography. It is an integrated LC designed for ease of use, performance, and reliability. It is well-suited for the analysis of drugs due to the highly precise retention times and peak areas, excellent detector linearity, and excellent injector linearity. This Application Note shows:

• Excellent retention time precision, with relative standard deviation (RSD) < 0.07 % for all 22 linearity levels.
• Excellent area precision, with RSD < 0.5 % for all 22 levels.
• Excellent injector linearity, with coefficient of correlation (R^2) > 0.9999. This study covers a wide range of 1 µL to 100 µL injected, and the solution concentration used was 100 ng/µL Tramadol.

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Introduction

For the analysis of drugs in routine QA/QC, it is very important to have highly precise, accurate, linear, and robust LC systems. The performance of the autosampler plays a major role in the accuracy of the analysis. The design of the Agilent 1120 Compact LC autosampler is well known for its accuracy and precision in injection. It delivers the needed data quality and is based on a proven robust design. In this Application Note, the linearity performance of the Agilent 1120 Compact LC autosampler is demonstrated via injections of Tramadol, an analgesic.

Figure 1
Agilent 1120 Compact LC.

Experimental

Equipment
The Agilent 1120 Compact LC system included:
- A gradient pump with low-pressure mixing
- An autosampler with vial tray (maximum injection volume is 100 µL)
- A column compartment for a column of up to 250 mm in length
- An Agilent TC (Typical Carbon load) C18(2) column, 250 x 4.6 mm, 5 µm
- A variable wavelength detector (VWD)

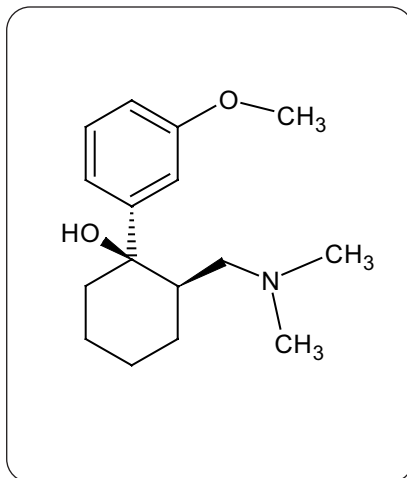The instrument was controlled by Agilent EZChrom Elite Compact software.

Figure 2
Chemical structure of Tramadol.

Sample preparation
Stock solution: A stock solution of 1 mg/mL Tramadol was prepared.

Linearity test sample (diluted stock solution): 1 mL of the stock solution was diluted to 10 mL, resulting in a concentration of 0.1 mg/mL (100 ng/µL).

Chromatographic parameters
A chromatographic method was developed to separate Tramadol and its potential impurities. This method was then used (with Tramadol alone) for the injector linearity tests. The conditions were:
- Sample: Tramadol
- Column: Agilent TC-C18(2), 250 x 4.6 mm, 5 µm
- Mobile phases: A = water + 0.2 % TFA, B = acetonitrile + 0.16 % TFA
- Flow rate: 1.2 mL/min
- Gradient: at 0 min 30 %B, at 9 min 85 %B, then hold the ratio for three more minutes
- Injection volume: varied from 1 µL to 100 µL
- Autosampler programmed with a wash vial
- Run time: 12 min
- Post time: 5 min
- Column oven: 30 °C
- VWD: 270 nm, peak width (PW) > 0.05 min
- Diluent / blank: 30:70 acetonitrile:water

Sequence table

The linearity test sample was injected as per the sequence table shown as table 1.

Results and discussion

Figure 3 shows an example chromatogram of Tramadol from the injector linearity test. The mobile phase contained trifluoroacetic acid as modifier, which improved retention and peak shape.

Serial #	Sample name	Injection volume (µL)	# Injections
1	Blank	5	2
2	Linearity Level:1	1	3
3	Linearity Level:2	3	3
4	Linearity Level:3	5	3
5	Linearity Level:4	10	3
6	Linearity Level:5	15	3
7	Linearity Level:6	20	3
8	Linearity Level:7	25	3
9	Linearity Level:8	30	3
10	Linearity Level:9	35	3
11	Linearity Level:10	40	3
12	Linearity Level:11	45	3
13	Linearity Level:12	50	3
14	Linearity Level:13	55	3
15	Linearity Level:14	60	3
16	Linearity Level:15	65	3
17	Linearity Level:16	70	3
18	Linearity Level:17	75	3
19	Linearity Level:18	80	3
20	Linearity Level:19	85	3
21	Linearity Level:20	90	3
22	Linearity Level:21	95	3
23	Linearity Level:22	100	3

**Table 1**
The sequence table.
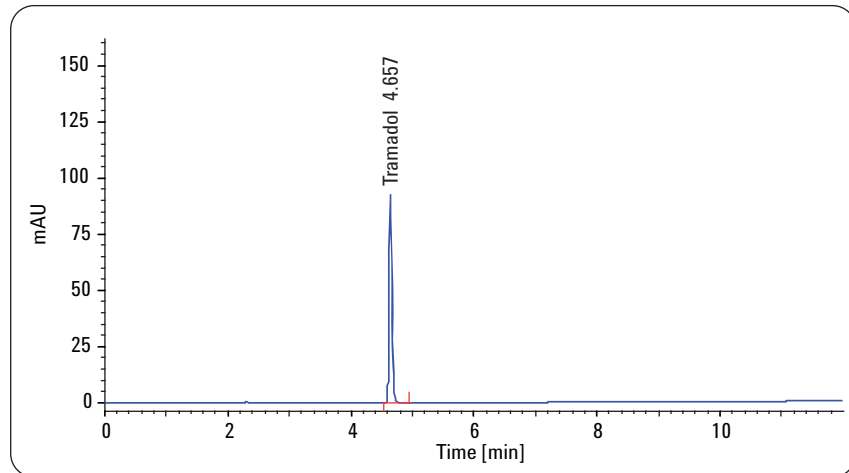
Figure 3
Chromatogram of Tramadol.

Table 2 lists the average peak heights and peak areas for each concentration that was injected.

The chromatographic overlay of all linearity levels is shown in figure 4.

| Sample name	On-column concentration (µg)	Peak height	Peak area
Linearity Level:1	0.1	157336	522936
Linearity Level:2	0.3	472170	1570002
Linearity Level:3	0.5	786250	2622929
Linearity Level:4	1.0	1553884	5244121
Linearity Level:5	1.5	2289525	7857707
Linearity Level:6	2.0	3000436	10471135
Linearity Level:7	2.5	3680418	13077010
Linearity Level:8	3.0	4338134	15685629
Linearity Level:9	3.5	4974238	18305228
Linearity Level:10	4.0	5584654	20913229
Linearity Level:11	4.5	6186702	23513327
Linearity Level:12	5.0	6750352	26119986
Linearity Level:13	5.5	7323479	28725172
Linearity Level:14	6.0	7800501	31103171
Linearity Level:15	6.5	8328025	33699927
Linearity Level:16	7.0	8833069	36264800
Linearity Level:17	7.5	9328439	38847902
Linearity Level:18	8.0	9811331	41437670
Linearity Level:19	8.5	10317715	44113978
Linearity Level:20	9.0	10734428	46673257
Linearity Level:21	9.5	11175523	49272540
Linearity Level:22	10	11601457	51871167

Table 2
**Tabulation of peak areas and heights.**
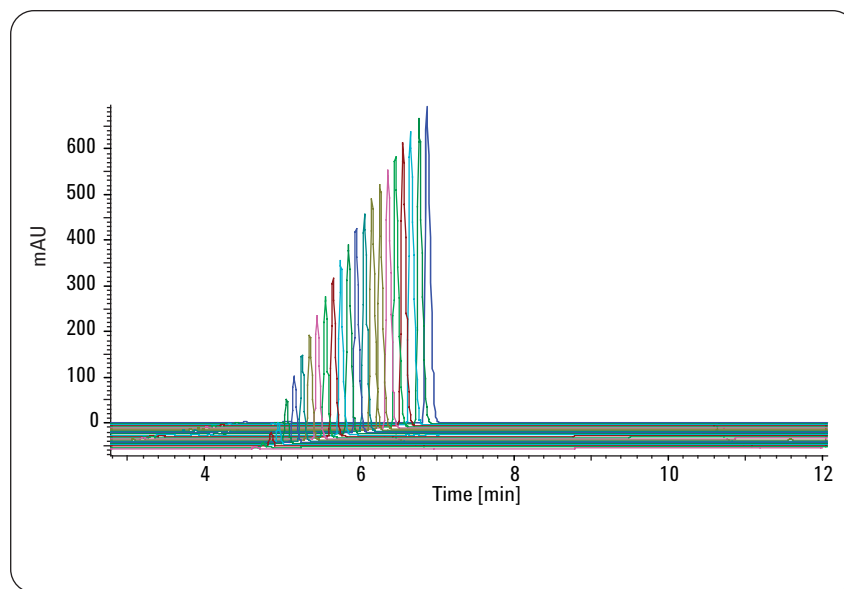
Figure 4
Chromatographic overlay of all linearity levels.

The linearity plot is displayed in figure 5.

Figure 6 shows the % RSD of retention times for each linearity level.
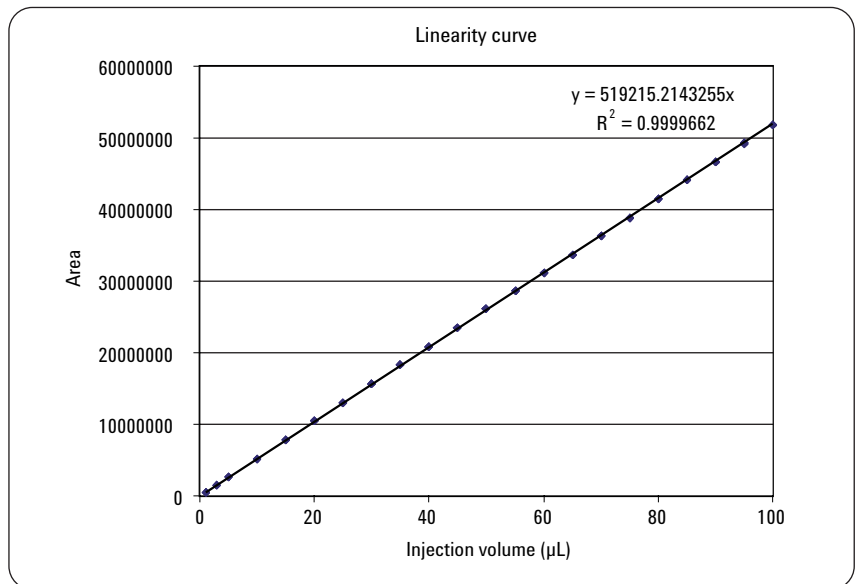
Figure 5
Injector linearity of the Agilent 1120 Compact LC using 1 μL to 100 μL of a solution of Tramadol. The observed linearity correlation (R2) is > 0.9999.

The equation shown in Figure 5:
$$y = 519215.2143255x$$
$$R^2 = 0.9999662$$
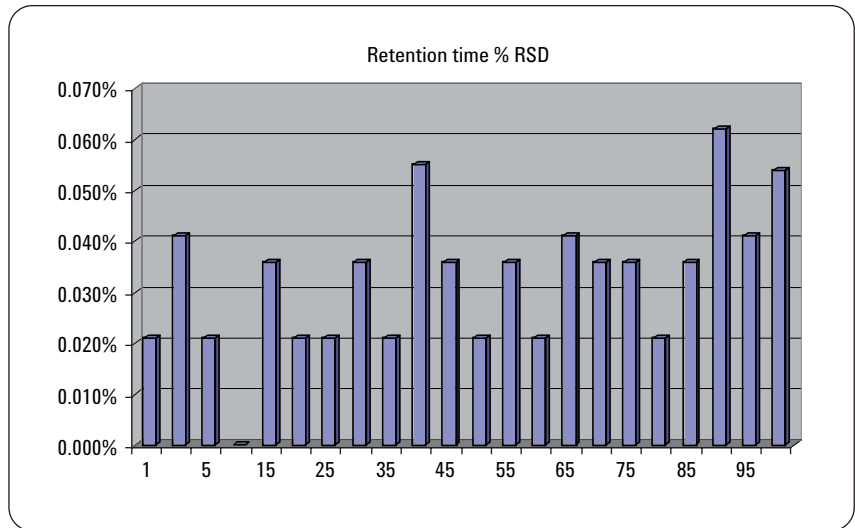
Figure 6
% RSDs for retention times. RSDs of all levels are within the limit of < 0.07 %.

Figure 7 shows the % RSD of the peak areas for each linearity level.

Conclusion

The autosampler that is integrated into the Agilent 1120 Compact LC gives excellent injection precision over a wide range of injection volumes (1 µL to 100 µL), and is a best choice for pharmaceutical QA/QC and drug discovery. Our study with Tramadol shows that the system is able to analyze this compound with high precision for retention times (< 0.07 % RSD) and for peak areas (< 0.5 % RSD).
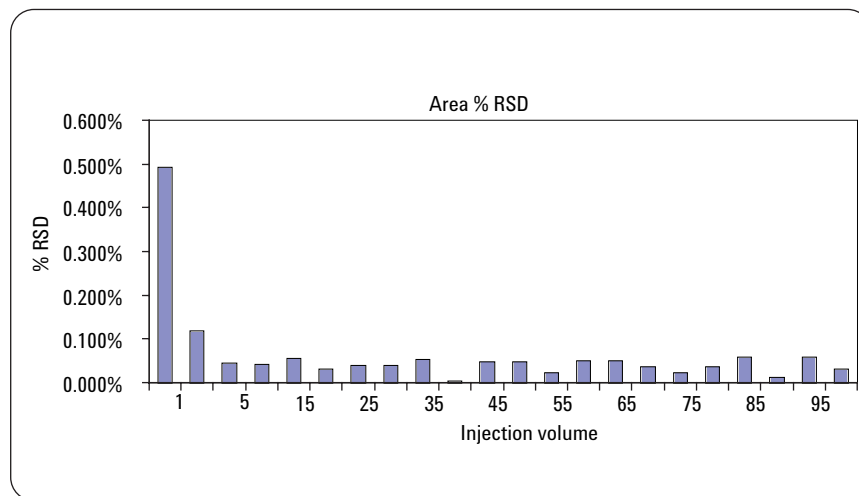
Figure 7
% RSDs of peak areas. RSDs of peak areas for all the levels are within the limit of < 0.5 %.

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