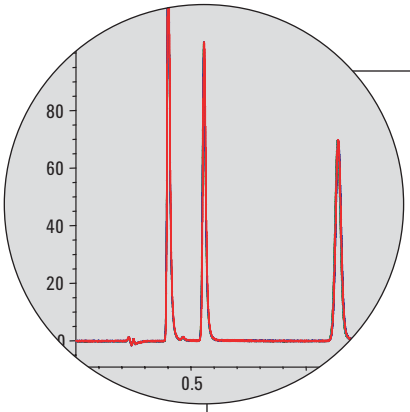
The Agilent 1200 Series high performance autosampler SL: Area precision, injection volume linearity, minimum accessible volume, carry-over

Technical Note



Introduction

A main performance criterion of any LC system is area precision, which is influenced primarily by the autosampler. The Agilent 1200 Series high performance autosampler SL is based on the so-called flow-through design. An injection valve switches the metering device in and out of the flow path. The metering device is switched out of the flow path when the sample is drawn. Then the injection valve is switched and the sample is injected onto the column by the flow provided by the pump. The metering device typically stays in the flow path during the complete run time and is therefore continuously flushed. The advantage of this type of injector is that precise injections over a wide injection volume range are possible without the necessity of hardware changes. Also, injection volume linearity is typically available over a wide injection volume range. Furthermore, no sample is wasted – the sample volume that is drawn is injected. In this Technical Note the area precision for fast gradient and isocratic applications is evaluated. Further, the minimum accessible volume and injection volume linearity is determined and experiments for providing lowest carry-over are performed.

Experimental

The Agilent 1200 Series Rapid Resolution LC system was used with the following modules:

- Agilent 1200 Series binary pump SL with vacuum degasser for applications using 1.8-µm particle columns up to 150 mm length and with internal diameters from 2.1 to 4.6 mm
- Agilent 1200 Series high performance autosampler SL for highest area precision
- Agilent 1200 Series thermostatted column compartment SL with wide temperature range from 10 degrees below ambient up to 100 °C
- Agilent 1200 Series diode-array detector SL for 80 Hz operation, including a new data protection tool
- ZORBAX SB C-18 columns with different internal diameters and lengths, packed with 1.8-µm particles

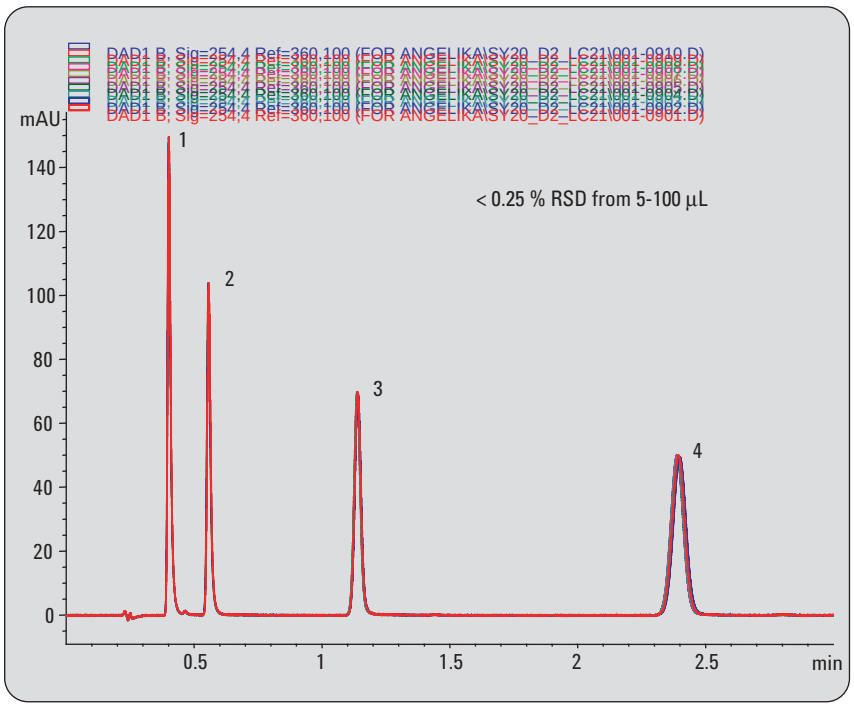


Figure 1
Overlay of 10 runs injecting 5 µL each.

Chromatographic conditions

Sample:	Agilent isocratic standard sample, 1 dimethylphthalate 0.15 wt %, 2 diethylphthalate 0.15 wt. %, 3 biphenyl 0.01 wt. %, 4 o-terphenyl 0.03 wt %
Column:	2.1 x 50 mm ZORBAX SB C-18, 1.8 µm, 600 bar
Mobile phase:	water/ acetonitrile = 60/40, isocratic
Flow rate:	0.5 mL/min
	DAD, 2 µL cell, 80 Hz, 2-mm slit, 254, 4 nm ref. 360/100
Column temp:	31.5 °C
Sample:	isocratic standard dissolved in mobile phase

Results and discussion

Precise injection is mandatory for good quantitative results in LC chromatography. The Agilent 1200 Series high performance autosampler SL is able to inject precisely over a wide injection volume range (figures 1 and 2)

In figure 1 an example is given for the precise injection of a 5-µL sample. The LC conditions are isocratic and the run time is only 3 minutes. Typically, the precision for injection volumes from 5 to 100 µL is about 0.25 % RSD. For volumes of 1-5 µL, variable, the precision is <1 % RSD (figure 2).

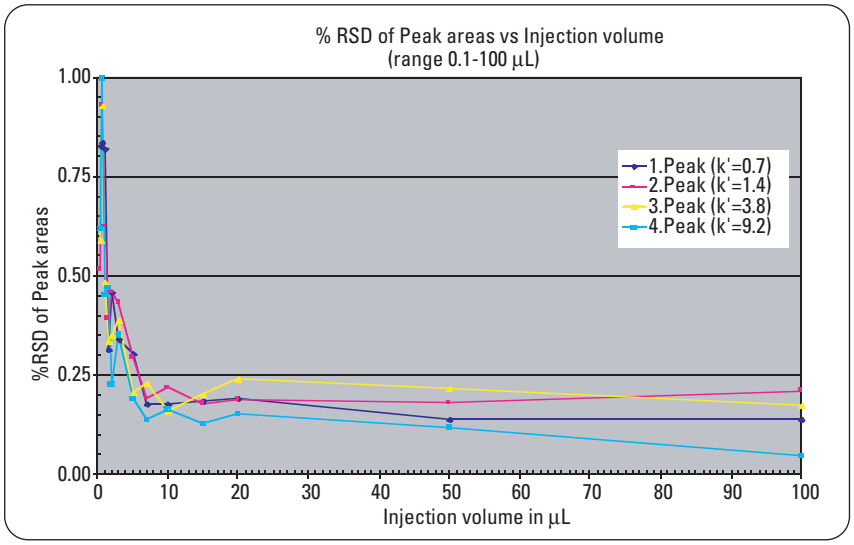


Figure 2
RSD of areas over an injection volume range from 0.1 to 100 µL. Conditions see figure 1.

Chromatographic conditions

Test sample	Set of 9 compounds, 100 ng/µL each, dissolved in water/ ACN (65/35) 1. Acetanilide, 2. Acetophenone, 3: Propiophenone, 4. Butyrophenone (200 ng/µL), 5.Benzophenone, 6. Valerophenone, 7. Hexanophenone, 8. Heptanophenone, 9. Octanophenone	
Solvent:	A = water, B = ACN	
Column:	2.1 x 50 mm ZORBAX SB C-18, 1.8 µm, 600 bar	
Temperature:	40 °C	
Flow:	0.7 mL/min	
Gradient:	0.00 min	35 %B
	1.30 min	95 %B
	1.60 min	95 %B
	1.61 min	35 %B
Stoptime:	1.60 min	
Posttime:	1.00 min	
Wavelength:	245 nm (8), Ref. 450 nm (100)	
Slit:	8 nm	
Peak width:	>0.0025 min (0.05 s response time), 80 Hz	
Spectra:	all, 190-500 nm, BW = 1 nm	
Injection volume: 0.5 µL, 1.0 µL, 3.0 µL		
Injector:	Overlapped injection Automatic delay volume reduction Sample flush out factor = 10 Needle wash = 5 s	

Figure 3 shows the area precision for a fast gradient application. The area precision was determined for injection volumes of 0.5 µL, 1.0 µL and 3.0 µL. After 10 repetitions the precision was always below 1 % RSD, even as low as 0.3 % RSD for the larger injection volume. Linearity of the injection volume range was tested injecting 375 ng of the "phenone mix" for each injection. The injection volume was varied but the injected amount was kept constant. As a result, the area counts should be the same for all injection volumes (figure 4).

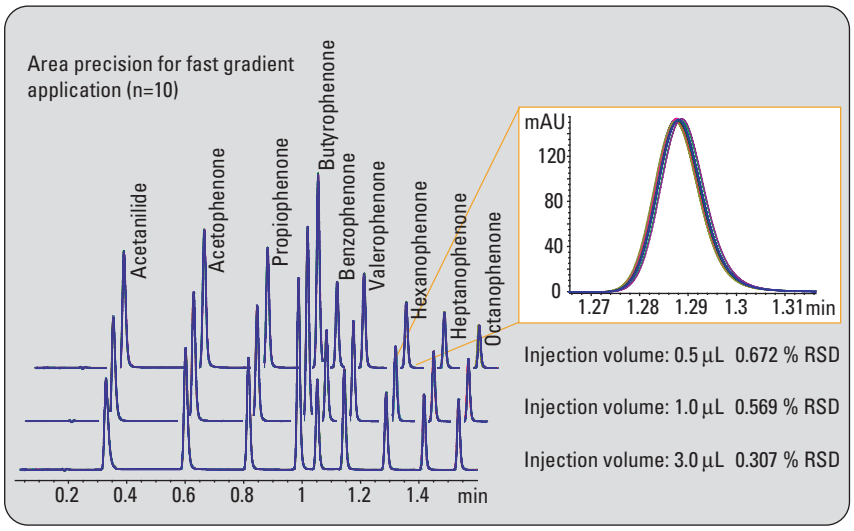


Figure 3
Gradient analysis of "phenone mix", overlay of 10 runs of 0.5 µL, 1 µL and 3 µL.

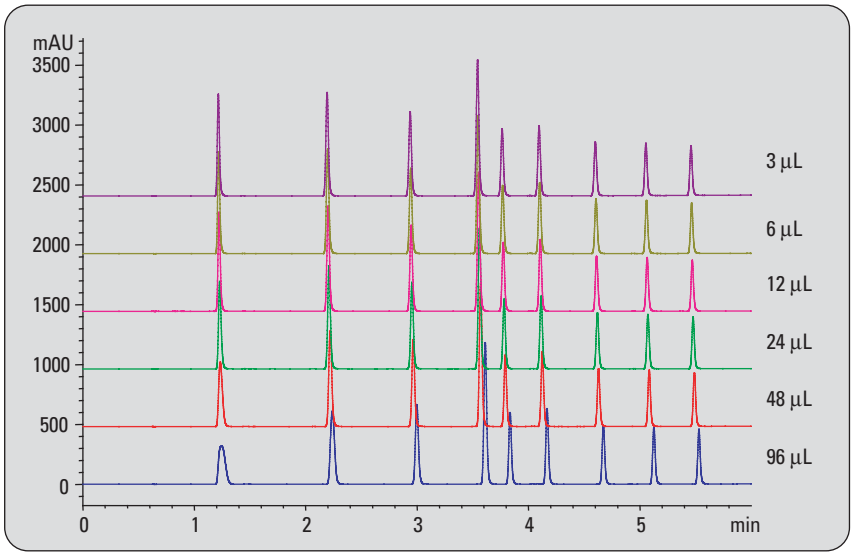


Figure 4
Overlay of phenone sample using the same amount of sample (375 ng/injection) with different injection volumes.

Chromatographic conditions

Test sample:	Set of 9 compounds, 100 ng/µL each, dissolved in water/ ACN (65/35) 1. Acetanilide, 2. Acetophenone, 3. Propiophenone, 4. Butyrophenone (200 ng/µL), 5. Benzophenone, 6. Valerophenone, 7. Hexanophenone, 8. Heptanophenone, 9. Octanophenone	
Column:	100 x 4.6 mm ZORBAX SB C-18, 1.8 µm for 600 bar operation	
Pump:	Solvent A=water B=ACN	
Gradient:	35 to 95 % B in 5 min, hold over 1 min, stop time 6 min, post time 2 min	
Flow rate:	1.5 mL/min	
Autosampler:	Injection volume:	3 to 96 µL
	Wash:	10 s for exterior of needle
Thermostatted column compartment:		
	Temperature: 50 °C	
Detector:	13-µL cell, 20 Hz data acquisition	
	Peak width:	<0.01 min
	Rate slit:	8 nm
	Signal:	245/10 nm, ref 360/80 nm

	Peak 1	Peak 2	Peak 3	Peak 4	Peak 5	Peak 6	Peak 7	Peak 8	Peak 9
3 µL	1199.9	1453.6	1210.3	1955.7	926.3	995.75	756.25	722.04	668.16
6 µL	1221.8	1479.6	1231.1	1986.8	942.78	1014.4	768.81	733.36	677.45
12 µL	1240	1495.5	1243.3	2005.9	955	1020.8	775.15	739.98	682.23
24 µL	1256.3	1518.7	1263.4	2037.2	967.32	1039.2	787.68	751.01	692.54
48 µL	1290.5	1566	1302.9	2105.7	996.73	1071.9	815.09	781.57	718.22
96 µL	1315.8	1590	1320.7	2134.3	1016.3	1089.2	829.7	794.05	735.51
average	1254.05	1517.233	1261.95	2037.6	967.405	1038.542	788.78	753.6683	695.685
s.d.	39.40477	47.63251	38.93977	63.61577	30.82364	32.68076	25.85424	25.87276	23.71682
% RSD	3.142201	3.139432	3.085683	3.122093	3.186218	3.146793	3.277751	3.432911	3.409132

Table 1
Area precision from 3 to 96 µL injection volume injecting 375 ng each.

The area precision was evaluated for the different injection volumes (table 1). As the same amount was always injected, the areas should be the same over the complete injection volume range. The first 2 to 3 peaks which elute isocratically broaden with increasing injection volume. The peaks which elute under gradient conditions keep peak shape and peak height.

The area precision is < 3.5 % RSD over the complete injection volume range.

Another important parameter is the minimum accessible volume, which strongly depends on the sample vial used. Small vials with conical bore and a volume as low as 100 µL are best suited to access even 1 µL out of 3 µL sample volume, (figure 5). To be able to draw 1 µL out of 3 µL it is necesary to activate the "well/vial bottom sensing" feature in the autosampler set up screen.

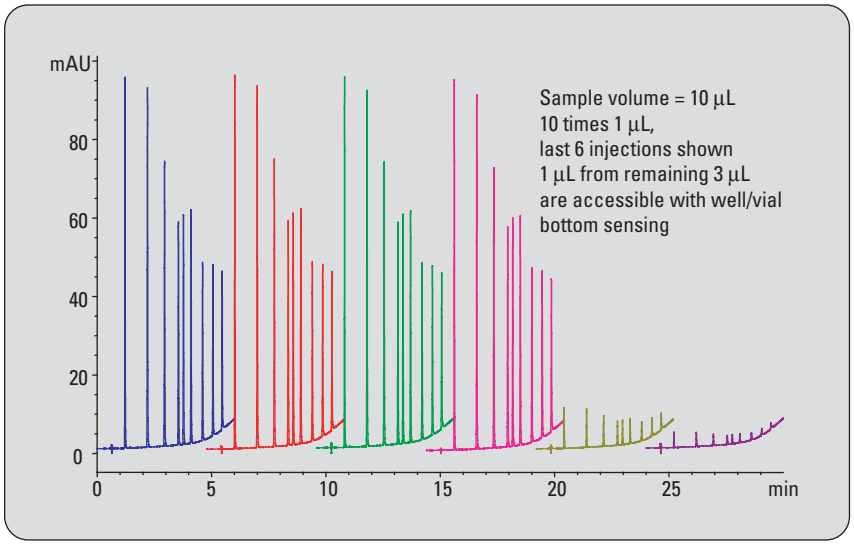


Figure 5
Last six consecutive 1-µL injections from a sample volume of 10 µL. Minimum accessible volume from a 100 µL conical glass vial is 1 µL of the remaining 3 µL using well/vial bottom sensing.

Chromatographic conditions

Test sample: Set of 9 compounds, 100 ng/uL each, dissolved in water/ ACN (65/35)
1. Acetanilide, 2. Acetophenone, 3. Propiophenone, 4. Butyrophenone (200ng/µL), 5. Benzophenone, 6. Valerophenone, 7. Hexanophenone, 8. Heptanophenone, 9. Octanophenone

Column: 100 x 4.6 mm ZORBAX SB C-18, 1.8 µm for 600 bar operation

Pump: Solvent A =water, B=ACN

Gradient: 35 to 95 % B in 5 min, hold over 1 min, stop time 6 min , post time 2 min

Flow rate: 1.5 mL/min

Autosampler: Injection volume: 1 µL
Wash: 10 sec for needle exterior
Well/vial bottom sensing

Thermostatted column compartment:
Temperature: 50 °C

Detector: 13-µL cell, 20 Hz data acquisition
Peak width: <0.01 min
Rate slit: 8 nm
Signal: 245/10 nm ref 360/80

Carry-over was tested using the standard setup for the Agilent 1200 Series well-plate sampler with exterior needle wash for 10 seconds, (figure 6).

The carry-over was found to be below the detection limit for the conditions used. After the injection of 3 rounds of 500 ng of sample unadulterated acetonitrile was injected. The peaks occurring in the blank run are due to impurities in the water used. For more information on how to minimize carry-over see reference 1.

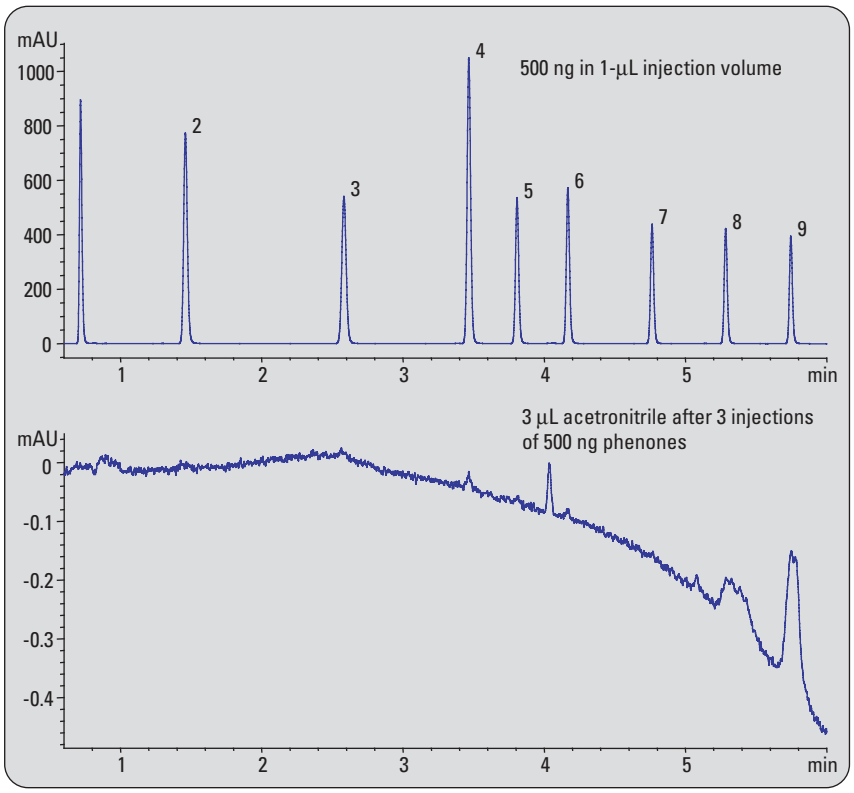


Figure 6
Carry over for "Phenone" sample, carry-over below LOD.

Chromatographic conditions

Test sample	Set of 9 compounds, 100 ng/uL each, dissolved in water/ ACN (65/35) 1. Acetanilide, 2. Acetophenone, 3. Propiophenone, 4. Butyrophenone (200 ng/µL), 5. Benzophenone, 6. Valerophenone, 7. Hexanophenone, 8. Heptanophenone, 9. Octanophenone
Column:	100 x 2.1mm ZORBAX SB C-18, 1.8 µm for 600 bar operation
Pump:	Solvent A =water, B=ACN
Gradient:	35 to 95 % B in 5 min, hold over 1 min, stop time 8min , post time 5 min
Flow rate:	0.6 mL/min
Autosampler:	Injection volume: 3 µL Wash: 10 sec for needle exterior
Thermostatted column compartment:	
	Temperature: 50 °C
Detector:	2-µL cell, 20 Hz data acquisition, Peak width: >0.01 min Rate slit: 8 nm, Signal: 245/10 nm ref 360/80 nm

Conclusion

The Agilent 1200 high performance autosampler SL provides excellent precision over a wide injection volume range. With an injection volume between 5 and 100 µL, the precision is typically <0.25 % RSD. Injection volume linearity is available from 3 µL up to 100 µL. The minimum accessible sample amount is 1 µL extracted from 3 µL using a conical 100-µL vial. The carry-over is due to the flow-through design and the external needle wash routine, typically < 0.01%.

Reference

1.

A.G.Huesgen, "Optimization of the Agilent 1100 Series well-plate autosampler for lowest carry-over using an optional injector purge kit", *Agilent Application Note, publication number 5989-3357EN*, **2005.**

www.agilent.com/chem/1200rrht

Published May 1, 2006
Publication Number 5989-5161EN

Agilent Technologies