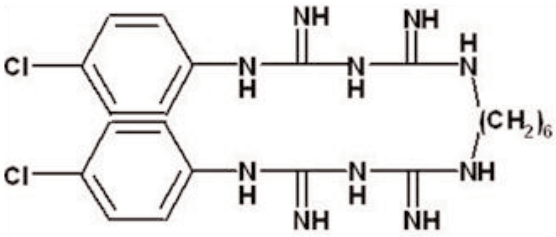
Performance characteristics of the Agilent 1200 Series high performance autosampler SL Plus

New design and materials for near zero carrryover

Technical Overview



Abstract

The new Agilent 1200 Series high performance autosampler SL Plus is based on a revised design and encompasses new parts, materials, cleaning procedures and a special surface treatment of all capillaries. The proven flow-through design was maintained to ensure a wide linear injection volume range and the possibility to inject small sample volumes without loss of sample. The new design has a small delay volume of 140 µL and helps to achieve:

- Lowest carryover, typically 0.001 – 0.004 %
- Highly precise areas

Agilent Equipment

- 1200 Series Rapid Resolution LC system
- 1200 Series high performance autosampler SL Plus
- 6140 quadrupole MS

Agilent Technologies

Introduction

During the last decade the sensitivity of UV and MS detectors has improved significantly. As a result, traces of compounds in the ppb and ppt ranges remaining in the LC system can cause carryover. Parts of the LC system that could be the cause of carryover include:

- Exterior and interior surfaces of injection needle
- Seat capillary
- Injection valve
- Fittings
- Interior surfaces of flow capillaries in contact with the sample
- Separation column

After the injection of a concentrated sample, carryover may become a severe problem. Any part of the LC system in contact with sample compounds can be the cause of carryover. The flow-through design of the Agilent 1200 Series autosamplers is capable of maintaining carryover below or close to the limit of detection for many compounds. Nevertheless, some compounds lead to more carryover than others and therefore require special precautions. Systems with PEEK tubing have demonstrated very low carryover effects for many of these difficult compounds. These systems typically exhibit carryover of about 0.005 %. However, PEEK has a typical upper pressure limit of 220 bars, making it unsuitable for use with sub-2-micron particle column materials that generate higher system backpressures.

Challenging carryover issues with the Agilent 1200 Series autosampler SL were addressed by adopting a completely new design. The most significant design changes were the development of a new injection needle, needle seat and fittings. Further, a new treatment was developed for all flow capillaries to passivate any active sites on the inner surfaces.
In this publication the performance of the new Agilent 1200 Series autosampler SL Plus is described relating to:

- Carryover
- Injection volume linearity
- Area precision

Experimental

Equipment

- The Agilent 1200 Series Rapid Resolution LC/MS system comprised the following modules with firmware revisions A.06.01 or higher:
- Agilent 1200 Series binary pump SL with degasser
- Agilent 1200 autosampler SL Plus
- Agilent 1200 Series thermostatted column compartment SL
- Agilent 1200 Series diode array detector SL
- Agilent 6140 quadrupole MS with ESI source operated in SIM mode
- Agilent ZORBAX RRHT 1.8 µm columns

Results and discussion

Design and functionality of the new autosampler

The Agilent 1200 Series SL Plus autosampler is based on the flow-through design illustrated in figures 1 and 2. The flow-through design offers a wide linear injection volume range and the possibility to inject small sample volumes without loss of sample.

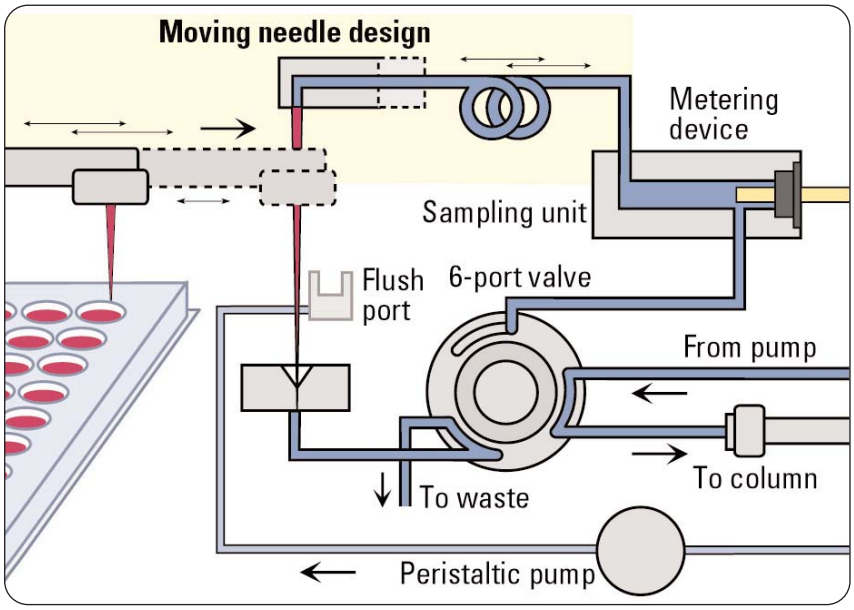


Figure 1
Aspiration of sampling from vial with flow path in bypass position.

Flushing and cleaning of the Agilent 1200 Series autosampler SL Plus to achieve near zero carryover

During the injection routine of the autosampler the sample loop, the inside of the needle, the seat capillary and the main channel of the injection valve are in the flow path and remain there throughout the duration of the run (figure 2). This means these parts are flushed continuously with mobile phase during the complete analysis. It is only during aspiration of the sample that the injection valve is switched out of the flow path (figure 1). In this position the pump effluent is led directly to the column.

Prior to injection the outside surfaces of the needle are washed with fresh solvent. This is achieved using the flush port of the autosampler and prevents contamination of the needle seat. The flush port of the autosampler is refilled with fresh solvent by a peristaltic pump that is installed in the autosampler housing. The flush port has a volume of about 680 µL and the pump delivers 6 mL/min. Setting the wash time to 10 seconds means the flush port volume is refilled more than once with fresh solvent, which is sufficient in most cases to clean the outside of the needle.

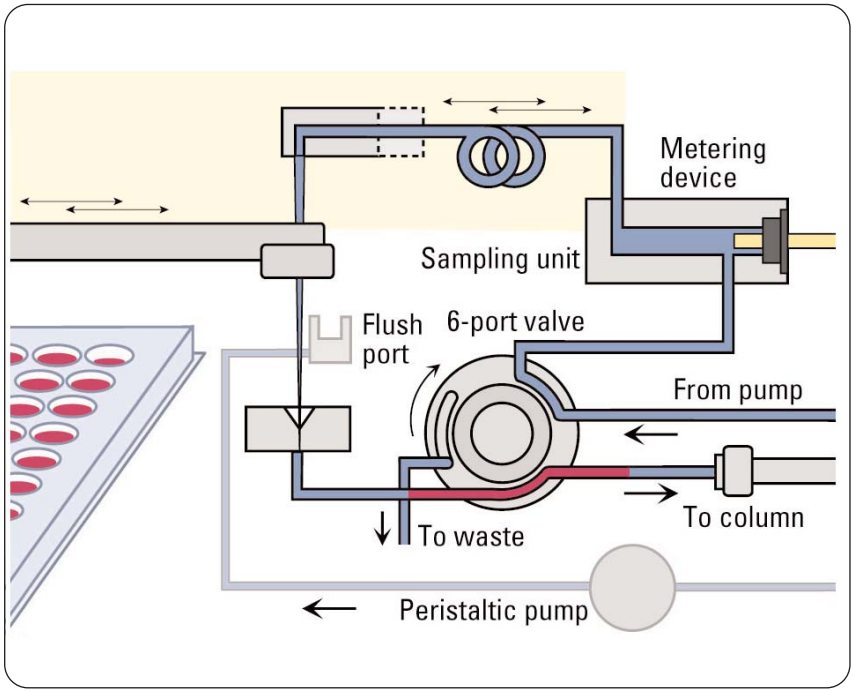


Figure 2
Sample injection with needle in flow path for duration of run.

Design changes in the new autosampler

The changes in the design of the new autosampler include:

- New injection needle
- New needle seat
- Smaller loop capillary behind the injection needle
- New low volume metering device

Further, the fitting between the needle and the loop capillary has been optimized and all capillaries have been specially treated. The combination of the cleaning procedures and the design changes have resulted in several positive effects:

- Significant lower carryover, typically < 0.001 and 0.004 % depending on the compound properties
- Significant lower delay volume of 140 µL
- Smaller injection volume range from 0.1 to 40 µL with enhanced-precision from 1 to 5 µL

Performance evaluation

Carryover behavior

The carryover behavior of the Agilent 1200 Series high performance autosampler SL Plus was evaluated using three different compounds. Chlorhexidine and beclomethsone are well known to cause severe carryover when injected at high concentration. The third compound, primidone, is less critical.

Chlorhexidine (figure 3) is an antimicrobial drug and is well known to show strong carryover, due to its chemical properties and over the years, it has often been used to evaluate carryover performance by many autosampler manufacturers. The free base dissolves well in methanol. It dissolves slowly in acidified water. For this evaluation the compound was dissolved in 0.1 % trifluoroacetic acid (TFA).

To evaluate the limit of detection (LOD) and the linear range of the method, a dilution series was set up and analyzed. Six solutions were prepared ranging from 2.4 pg to 240 ng (table 1).

To evaluate carryover, the Agilent 6140 MS was used in selected ion mode (SIM). The target mass was 505.3 in scan mode. The limit of detection (LOD) was found to be about 0.6 pg with a signal-to-noise ratio of 2. The LOD calculation was based on results obtained from the injection of 2.4 pg of chlorhexidine. The MS results were linear from 2.4 pg to 2400 pg with a coefficient of correlation of 0.99999. This range was used to evaluate the system carryover. The carryover was evaluated using the amount of compound determined in a blank run following an injection of a high amount of coumpond. The low end of the calibration table was used for this determination. This amount was then set in relation to the high amount injected in the previous run. Figure 4 shows the chlorhexidine and blank chromatograms superimposed.

After an injection of 240 ng of chlorhexidine, the carryover was found to be 0.0028 %, equivalent to 8 pg.

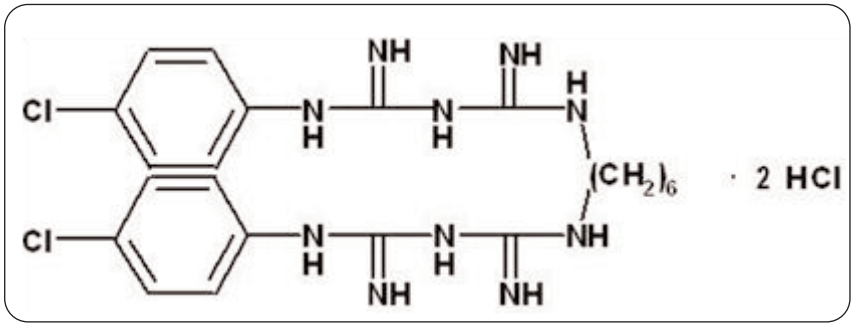


Figure 3
Chemical structure of chlorhexidine.

Chlorhexidine concentration	ng/µL	ng/µL	ng/µL	pg/µL	pg/µL	pg/µL
Dilution series	240	24	2.4	240	24	2.4

Table 1
Concentration range used for LOD and linearity test.

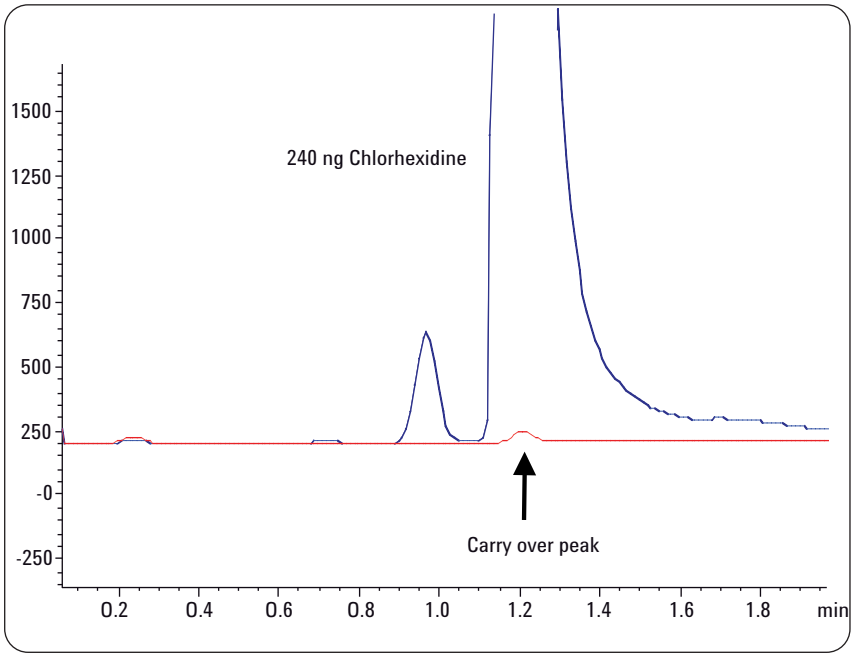


Figure 4
Overlay of 240 ng and blank injections.

Chromatographic conditions:

Column:	ZORBAX SB C18, 2.1x 50 mm, 1.8 µm	
Sample:	Chlorhexidine, dissolved in water + 0.1 % TFA	
Mobile Phase:	A: Water + 0.05 % TFA, B: Acetonitrile + 0.045 % TFA	
Flow rate:	0.5 mL/min	
Gradient:	30 to 60 %B after 3min	
Injection volume:	1 µL, external needle wash with water + 0.1%TFA, draw and inject speed 100 µL/min	
Column temp.:	50 °C	
Detection:	API-ES positive:	SIM ion 505.30
	Fragmentor:	70
	Gas temperature:	250 °C
	Drying gas:	12 L/minVcap: 3000 V

Beclomethasone (figure 5) is a synthetic corticosteroid and has anti-inflammatory properties. Beclomethasone dipropionate is a pro-drug to beclomethasone and was used as test compound because it is also known to cause severe carryover. It dissolves well in Methanol.

To evaluate the limit of detection (LOD) and the linear range of the method, a dilution-series was set up and analyzed. Six solutions were prepared, ranging from 3 pg per 3 µL to 300 ng per 3 µL, see table 2. The LOD was found to be about 0.35 pg with a signal-to-noise ratio of 2. The calculation was based on the results obtained from the injection of 3 pg of Beclomethasone dipropionate. The MS results were linear from 3 pg to 300 pg with a coefficient of correlation of 0.99929. This range was used to quantitfy the amount of carryover.

To evaluate the carryover the Agilent 6140 MS was used in SIM mode. The target mass was 521.4 in scan mode. Carryover was found to be about 0.0022 %, equivalent to about 6.5 pg, (figure 6).

Chromatographic conditions:

Column:	ZORBAX SB C18, 2.1 x 50 mm, 1.8 µm	
Sample:	Beclomethasone dipropionate dissolved in methanol	
Mobile phase:	A: Water + 0.05 % TFA B: Acetonitrile + 0.045 % TFA	
Flow rate:	0.5 mL/min	
Gradient:	50 to 80 %B after 3 min	
Inj. volume:	3 µL, external needle wash with methanol, draw and inject speed 100 µL/min	
Column temp.:	50 °C	
Detection:	API-ES positive:	SIM ion 521.2
	Fragmentor:	70
	Gas temperature:	250°C
	Drying gas:	12 L/min
	Vcap:	3000 V

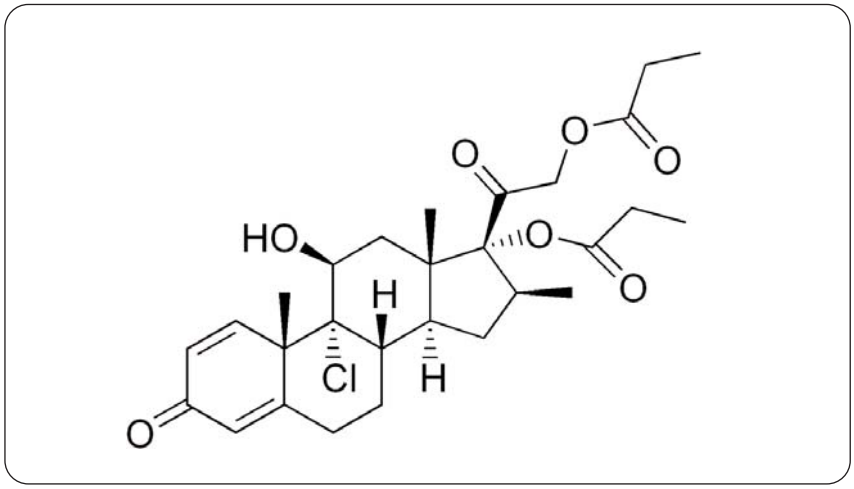


Figure 5
Chemical structure of beclomethasone dipropionate.

Beclomethasone diproprionate concentration	ng/3 µL	ng/3 µL	ng/3 µL	pg/3 µL	pg/3 µL	pg/3 µL
Dilution series	300	30	3	300	30	3

Table 2
Concentration range used for LOD and Linearity test.

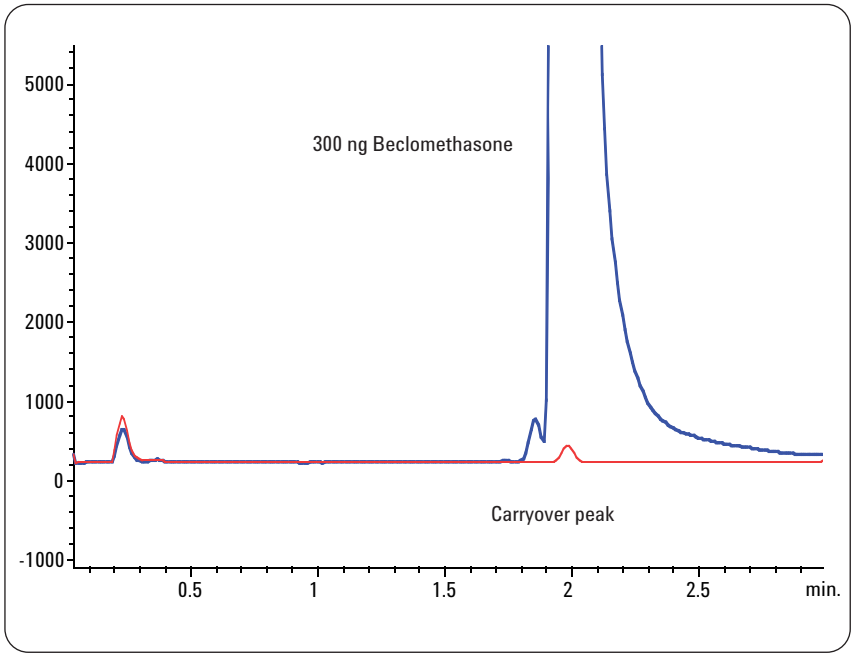


Figure 6
Overlay of highly concentrated sample with blank run.

Primidone is an antiepileptic drug which was used quite frequently in the past. It is a small molecule with a molecular weight of 219.1, (figure 7). To evaluate the limit of detection (LOD) and the linear range of the method, a dilution-series was set up and analyzed. Six solutions were prepared ranging from 3 pg up to 300 ng (table 3). The limit of detection (LOD) was found to be about 7 pg with a signal-to-noise ratio of 2. The calculation was based on results obtained from the injection of 30 pg of primidone. The MS results were linear from 30 pg to 300 pg with a coefficient of correlation of 1.00000. This range was used to quantify the amount of carryover. To evaluate carryover the Agilent 6140 MS was used in SIM mode. The target mass was 219.1 in scan mode. The carryover was found to be less than the LOD, (figure 8).

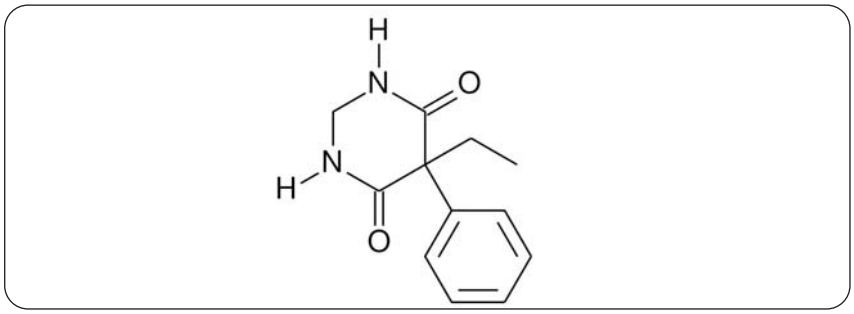


Figure 7
Chemical structure of Primidone.

Primidone concentration	ng/3 µL	ng/3 µL	ng/3 µL	pg/3 µL	pg/3 µL	pg/3 µL
Dilution series	300	30	3	300	30	3

Table 3
Concentration range used for LOD and linearity test.

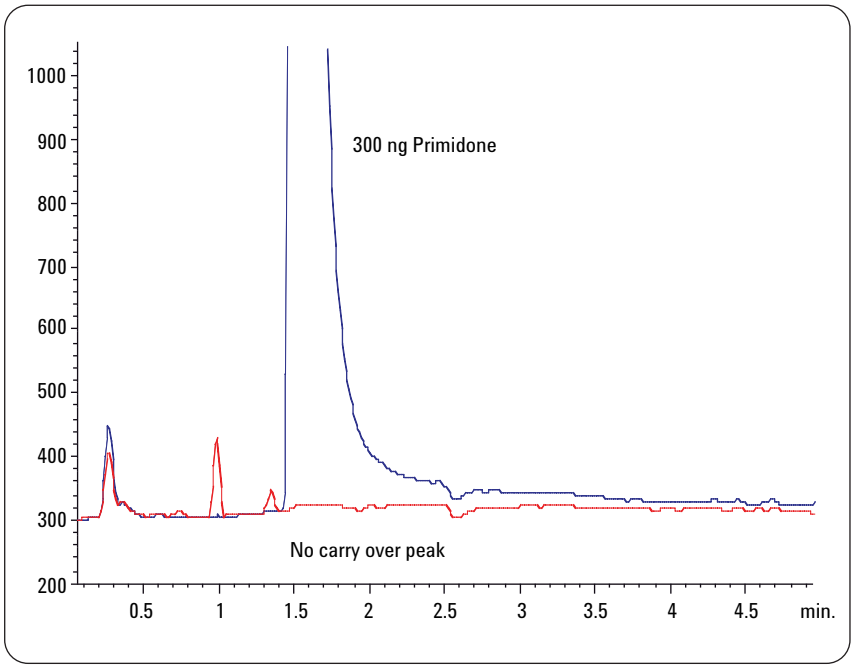


Figure 8
Overlay of high concentration and blank run – carryover is less than the LOD.

Recommendations for near zero carryover

To ensure lowest carryover, observe the following recommendations:

- Always use the autosampler with the injection valve in main-pass position.
- Flush the exterior of the needle with an appropriate solvent. The flush time should be a minimum of 10 s.
- Reduce the draw speed to 10 µL/min, if possible.
- Use Agilent capped 2 ml vials (order number 5182-0556)
- Use acidic mobile phases for basic compounds.

Chromatographic conditions:

Column:	ZORBAX SB C18, 2.1 x 50 mm, 1.8 µm	
Sample:	Primidone dissolved in water + 0.1 % TFA / methanol, 1:9	
Mobile phase:	A: Water + 0.05 % TFA, B: Acetonitrile + 0.045 % TFA	
Flow rate:	0.5 mL/min	
Gradient:	10 %B for 0.1 min, then 10 to 80 %B after 5 min	
Injection volume:	3 µL, external needle wash with methanol, draw and inject speed 10 µL/min	
Column temperature:	50 °C	
Detection:	API-ES positive:	SIM ion 219.1
	Fragmentor:	70
	Gas temperature:	250 °C
	Drying gas:	12 L/min
	Vcap:	3000 V

Precision for different injection volumes

Injection volumes depend on the concentration of samples and a wider injection volume range of an autosampler is better for different sample concentrations. For high performance autosamplers it is important to achieve good precision over the complete injection volume range. The injection volume for the Agilent 1200 Series autosampler SL Plus ranges from 0.1 up to 40 µL without the need to change hardware. Figure 9 shows an example chromatogram that is representative for the compounds and the conditions used for the evaluation of precision and injection volume linearity. This example was chosen because it is close to real-life applications with non-ideal baseline and peaks. Further, a concentration was selected that is in the low area count range to demonstrate that even under non-ideal conditions the autosampler and the integrator are able to deliver excellent results.

Table 4 shows the results for precision at low area counts for different injection volumes. The precision was measured for 0.5, 1, 2, 4, 8, 16 and 32 µL injection volumes. Six injections were evaluated for each injection volume. For peak heights between 2 and 12 mAU the precision for injection volumes from 0.5 to 4 µL is typically below 1 % RSD.

Linearity of injection volume

Good linearity in the low and high microliter injection volume range is another demand that should be met by a high performance autosampler. Linearity of the injection volume range was tested by injecting 0.5, 1, 2, 4, 8, 16 and 32 µL. The injection volume was varied but

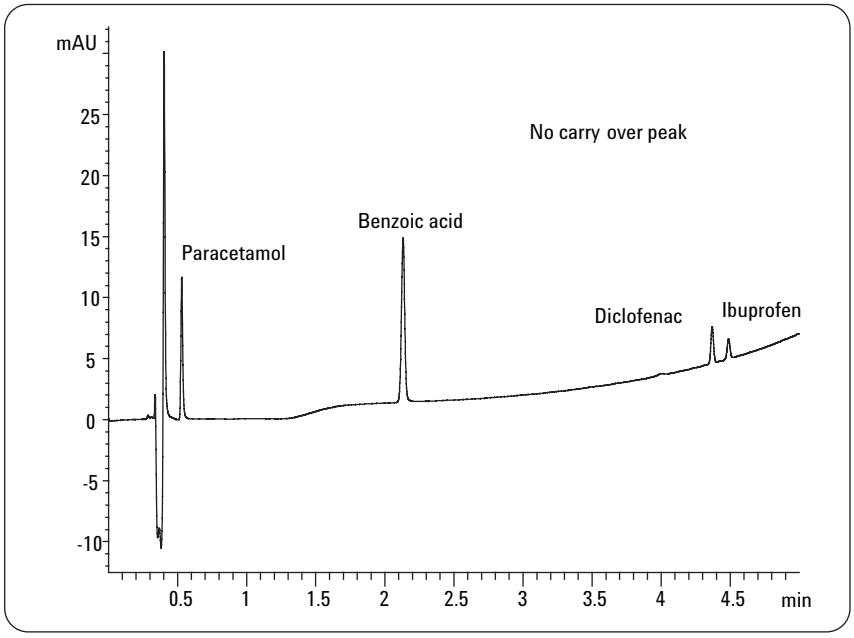


Figure 9
Example chromatogram used for the evaluation of precision and injection volume linearity.

Chromatographic conditions:

Column:	ZORBAX SB C18, 4.6 x 50 mm, 1.8 µm
Sample:	Paracetamol, benzoic acid, diclofenac and ibuprofen in water + 0.1 % TFA / methanol, 1:1
Mobile Phase:	A: Water + 0.05 % TFA, B: Acetonitrile + 0.045 % TFA
Flow rate:	1.5 mL/min
Gradient:	15 %B for 0.5 min, then 15 to 80 %B at 5 min
Injection volumes:	0.5, 1, 2, 4, 8, 16, 32 µL, external needle wash with Methanol, draw and inject speed 50 µL/min
Column temperature:	50°C
Detection:	230/16 nm, reference 360/100 nm, peak width > 0.03 min, slit 4 nm

Compound	Area RSD of 0.5 µL inj. vol. (%)	Area RSD of 1 µL inj. vol. (%)	Area RSD of 2 µL inj. vol. (%)	Area RSD of 4 µL inj. vol. (%)	Area RSD of 8 µL inj. vol. (%)	Area RSD of 16 µL inj. vol. (%)	Area RSD of 32 µL inj. vol. (%)
Paracetamol Height ~ 10 mAU	0.68	0.34	0.42	0.15	0.11	0.16	0.12
Benzoic acid Height ~ 12 mAU	0.18	0.39	0.25	0.20	0.12	0.24	0.19
Diclofenac Height ~ 3 mAU	0.23	0.59	0.91	0.54	0.55	0.40	0.44
Ibuprofen Height ~ 2 mAU	1.35	0.78	1.24	1.00	1.15	0.22	0.51

Table 4
Area precision of different injection volumes.

the injected compound amounts were kept constant. As a result, the area counts should be the same for all injection volumes. Table 5 shows the precision over the complete injection volume range is less than 3.8 % even at low area counts.

Figure 10 shows the linearity over the complete injection volume range from 0.5 up to 32 µL. For ibuprofen and the other tested compounds the response factors are within a ±3 % range.

Conclusion

The new Agilent 1200 Series high performance autosampler SL Plus is based on a revised design and encompasses new parts, materials and a special surface treatment of all capillaries. An Agilent 1200 Series Rapid Resolution LC system and an Agilent 6140 quadrupole mass spectrometer in selected ion mode (SIM) were used for all the carryover experiments. For compounds like primidone the carryover was found to be zero. For compounds such as chlorhexidine and beclomethasone, which are known to cause excessive carryover, the carryover was in the range of 0.002 to 0.004 %. The area precision for low injection volumes is typically less than 1 % or better even for low area counts. The measured injection volume linearity was within a ±3 % range for the response factors.

Injection volume	Paracetamol Average amount	Benzoic acid Average amount	Diclofenac Average amount	Ibuprofen Average amount
0.5	18.94784	22.89175	8.408343	12.72166
1	18.96998	23.15204	8.17972	12.45865
2	19.10388	23.53893	8.264042	12.46199
4	19.30402	24.0841	8.496533	12.43245
8	19.37102	24.40254	8.734967	12.34102
16	19.29554	24.81547	8.92029	12.20147
32	19.44944	25.39444	8.849168	11.92365
Average	19.20596	24.0399	8.550438	12.36298
SD	0.198704	0.906207	0.289434	0.249002
RSD	1.034597	3.769595	3.38502	2.014092

Table 5
Precision of amounts over an injection volume range from 0.5 to 32 µL..

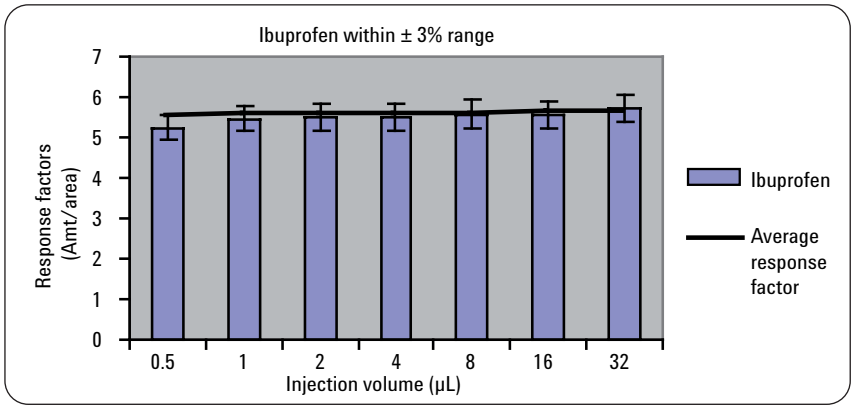


Figure 10
Response factors within ±3 % range for ibuprofen from 0.5 up to 32 µL injection volume.

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