Configuring and setting up the Agilent 1100 Series well-plate sampler for optimum performance

Technical Note

Introduction

Autosamplers have become an indispensable tool in HPLC. To benefit from the implemented features it is of utmost importance to be familiar with all the control functions. In this Technical Note we demonstrate how to set up the Agilent 1100 Series well plate sampler for optimum performance.

This Technical Note discusses how to

- configure the well plates,
- set up the control functions,
- set up lowest carry-over,
- set up fast cycle times,
- set up highest precision for areas,
- set up optimum linearity, and
- work at high pH.

This application has been verified using an Agilent 1200 Series LC system, and showed comparable or even better performance.

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Instrumentation

The following Agilent Series 1100 instrumentation was used for all experiments:

- Agilent 1100 Series high pressure gradient pump with lowest delay volume for fast gradient analysis,
- Agilent 1100 Series micro vacuum degasser for optimum baseline stability and fast solvent change,
- Agilent 1100 Series well-plate sampler with fast injections, overlapped injection mode for shortest cycle times and bypass position of autosampler for lowest system delay volume. Optionally, the Agilent 1100 Series autosampler can be equipped with a sample thermostat for cooling and heating. 96 well-plates and 96 deep-well-plates can be used as well as 384 well-plates and 2-ml vials. It is also possible to use 96 well-plates with glass inserts and caps. Closing mats are available.
- Agilent 1100 Series thermostatted column compartment for highest stability from 10° C below ambient up to 80° C,
- Agilent 1100 Series diode array detector with fast sampling rate and for additional spectral information,
- Agilent ChemStation Plus for instrument control, data handling, sample tracking and fast automated reporting and batch reviewing, and
- Agilent ChemStore C/S, data base module of Agilent ChemStation Plus, for data archiving and reviewing data independently from the Agilent ChemStation.

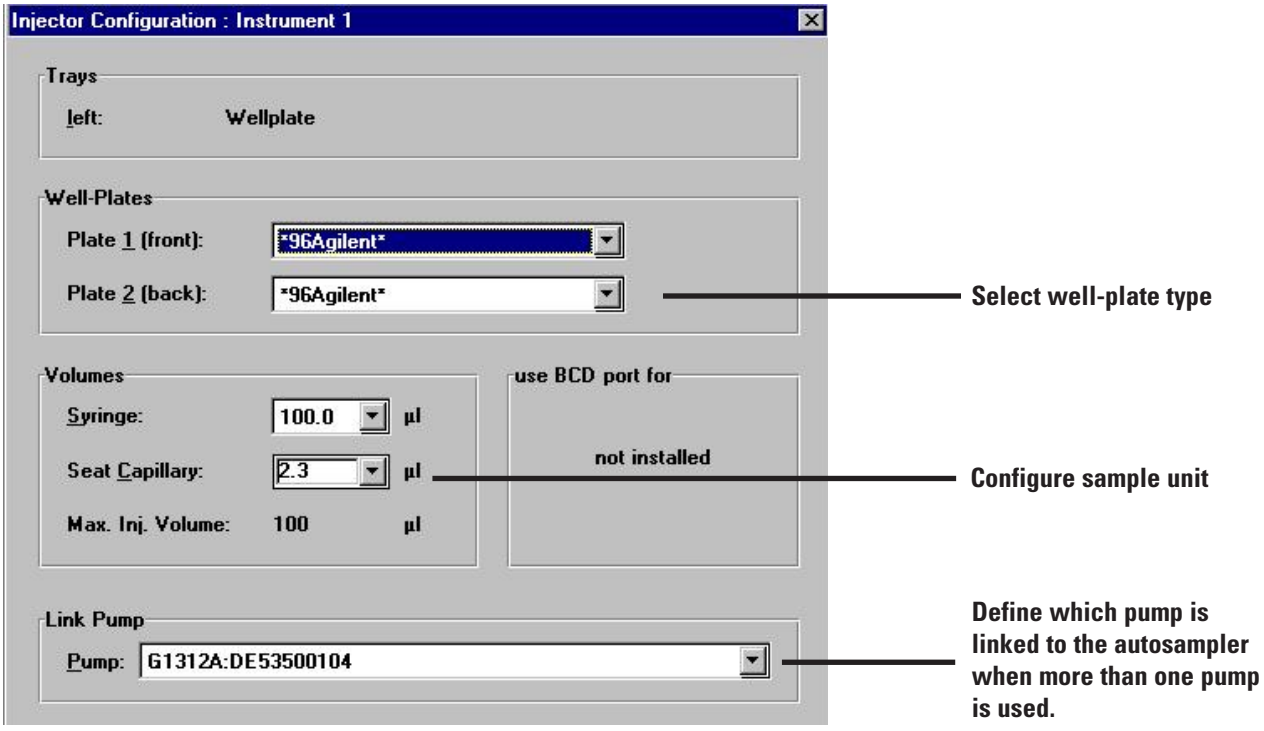


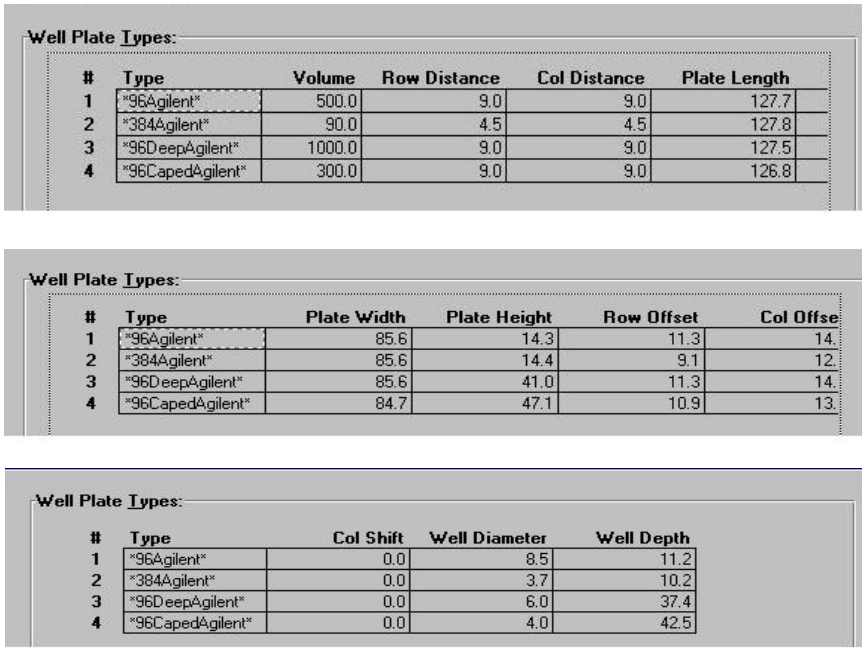
Figure 1
Configuring the well-plate sampler

Results and discussion

Configuring the Agilent 1100 Series well-plate sampler and customizing well-plates

In the configuration screen of the well-plate sampler three entries are necessary (figure 1). The preconfigured well-plates allow selection of well-plates directly from the drop down list. Both front and back plate can be configured independently. Syringe and seat capillary volume is predefined and must only be entered if one or both parts are changed for different volumes. The entry at the bottom of the screen is needed to define the pump that is linked to the autosampler, if more than one pump is used in the system. In this example the drop down menu contains one pump identification, which has to be selected. This entry is needed because with delay volume reduction or overlapped injection mode the time for switching the injection valve can be calculated automatically, based on the flow rate and on the injection volume. To combine the flow rate into these calculations the pump which is linked to the autosampler must be known.

Not all users will use predefined Agilent well-plates. In this case the well-plates have to be configured in the *Configure Well-plate Types* screen (figure 2). A series of parameter values in millimeters has to be entered to ensure that the needle finds the center of the wells. In most cases the parameters are available from the well-plate vendor. Otherwise the parameters have to be measured.



Well Plate Types:

#	Type	Volume	Row Distance	Col Distance	Plate Length
1	*96Agilent*	500.0	9.0	9.0	127.7
2	*384Agilent*	90.0	4.5	4.5	127.8
3	*96DeepAgilent*	1000.0	9.0	9.0	127.5
4	*96CapedAgilent*	300.0	9.0	9.0	126.8

Well Plate Types:

#	Type	Plate Width	Plate Height	Row Offset	Col Offse
1	*96Agilent*	85.6	14.3	11.3	14.
2	*384Agilent*	85.6	14.4	9.1	12.
3	*96DeepAgilent*	85.6	41.0	11.3	14.
4	*96CapedAgilent*	84.7	47.1	10.9	13.

Well Plate Types:

#	Type	Col Shift	Well Diameter	Well Depth
1	*96Agilent*	0.0	8.5	11.2
2	*384Agilent*	0.0	3.7	10.2
3	*96DeepAgilent*	0.0	6.0	37.4
4	*96CapedAgilent*	0.0	4.0	42.5

Figure 2
Configuring well-plates

Setting up autosampler parameters

All parameters needed to set up a chromatographic method for the well-plate sampler are included in the *Setup Injector s*creen, shown in figure 3.

First select a value for the *Injection Volume*, which is then valid for all single runs and for all sequence runs, if not otherwise specified in the sequence table. Next, the injection type has to be selected. In figure 3 *Injection with Needle Wash* was chosen. In the next section it is possible to exclude the autosampler volume from the system delay volume to reduce run and equilibration times. If low flow rates have to be used, for example, in microbore analysis, cycle times can be reduced significantly by excluding the autosampler delay volume from the system delay volume. To achieve this, the injection valve is switched into the bypass mode after the sample is flushed onto the column. The time needed to flush the sample onto the column is calculated by the instrument and is based on the injection volume and the flow rate.

Select *Enable Overlapped Injection* for high sample throughput. This means the next sample is drawn and kept in the sample loop before the previous run has ended. If this run is stopped then the sample which is kept in the sample loop is injected.
The time at which the injection valve is switched into the bypass mode and the next sample is drawn can be set using *When Sample is Flushed Out* or by entering a user-selectable time

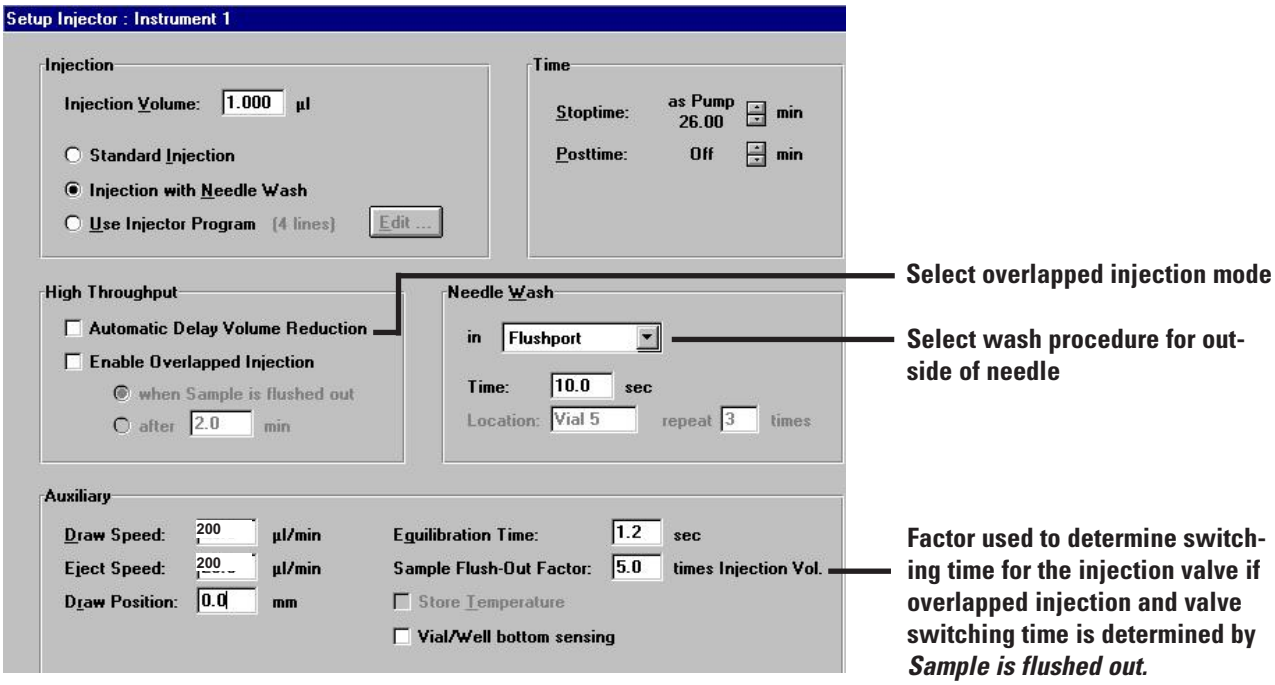


Figure 3
Setting autosampler parameters

value. The time when the valve is switched is calculated based on the flow rate and the injection volume to ensure that the sample is completely flushed onto the column. With the *Sample Flush Out Factor* this time can be prolonged or shortened.

If the time for switching the injection valve into the bypass mode is entered by the user the following equation can be used to ensure that the sample is able to reach the column.

$$\text{Wait time} = \frac{6\ (\text{inj. vol.} + 5\ \mu\text{l})}{\text{flow rate}}$$

Next, the wash procedure for the needle outside can be selected. The outside of the needle can be washed using a wash vial in a specific *Location* or the needle can be washed using *Flushport*. The flushport is located above the needle seat and a peristaltic pump delivers the wash solvent. The flush port has a volume of 0.68 ml and the peristaltic pump delivers 6 ml/min. This means in 10 seconds the flush port volume is refilled more than once with fresh solvent. This is sufficient for most applications. If the flushport is selected, the user can determine how long the needle outside is washed with fresh solvent.

Entries for the following parameters are only needed in special cases. Change of *Draw* and *Eject Speed* of the metering device to higher value can be done if large injection volumes are injected and cycle times should be reduced. But it has to be considered that when setting draw speed to its limit of 1 ml/min the precision of areas will be worse than with the default value. In case of a viscous sample the draw speed can be lowered to ensure bubble-free sampling.

Draw Position determines how deep the needle should slip into a well of a micro-titer plate or a vial. The default value (0 mm) should only be changed after testing how much liquid remains non-accessible in the well/vial using the default value.
The function *Vial/Well Bottom Sensing* is used to let the needle find the bottom of a well or a vial. Having found the bottom the needle is drawn back 1 mm. To be able to draw even small amounts of sample the *Draw Position* of the needle can now be set such that it touches nearly the bottom. Another parameter, which has to be discussed, is *Equilibration Time*. This is the time the needle remains in the well/vial after the sample is drawn. If a highly viscous sample is used, the time should be prolonged to ensure that no air bubbles are sucked into the needle when drawn out of the sample liquid. The last parameter *Store Temperature* inidcates that the actually set temperature of the sample thermostat is saved in the method.

Setting up autosampler parameters to run a sequence

Figure 4 shows the *Sequence Table* set up screen and a part of the *Method and Run Control* screen with two configured 96 well-plates. The sequence table can be set up manually or using *Insert Location Range*. Sequence lines can be added by “copy and paste”. In addition, the well-plate names *Plate 1 ID* and *Plate 2 ID* can be filled in (figure 5). These entries are saved with raw data and can be used for Agilent ChemStore reports, if the Agilent ChemStation Plus is used with the ChemStore C/S data base module.

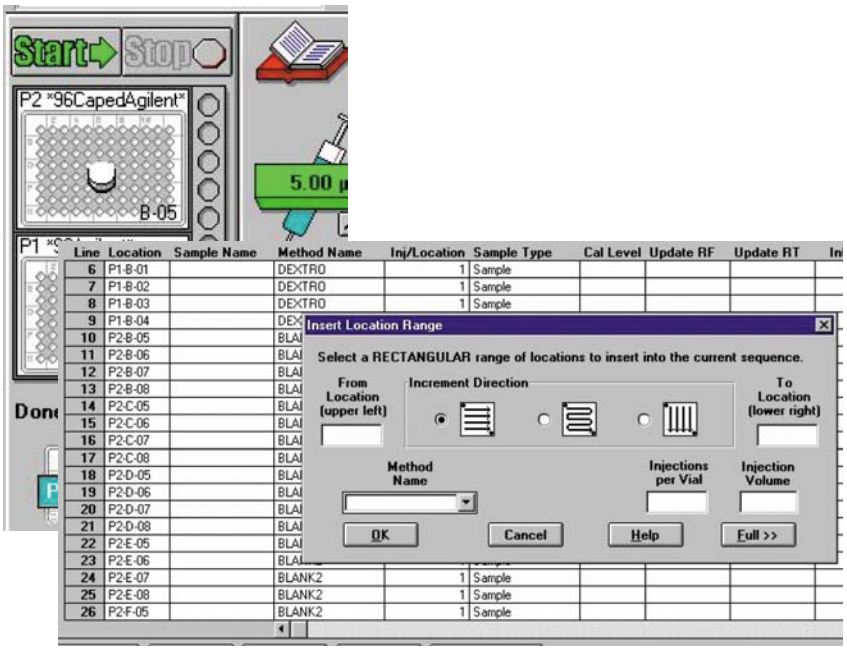


Figure 4
Setting sequence parameters

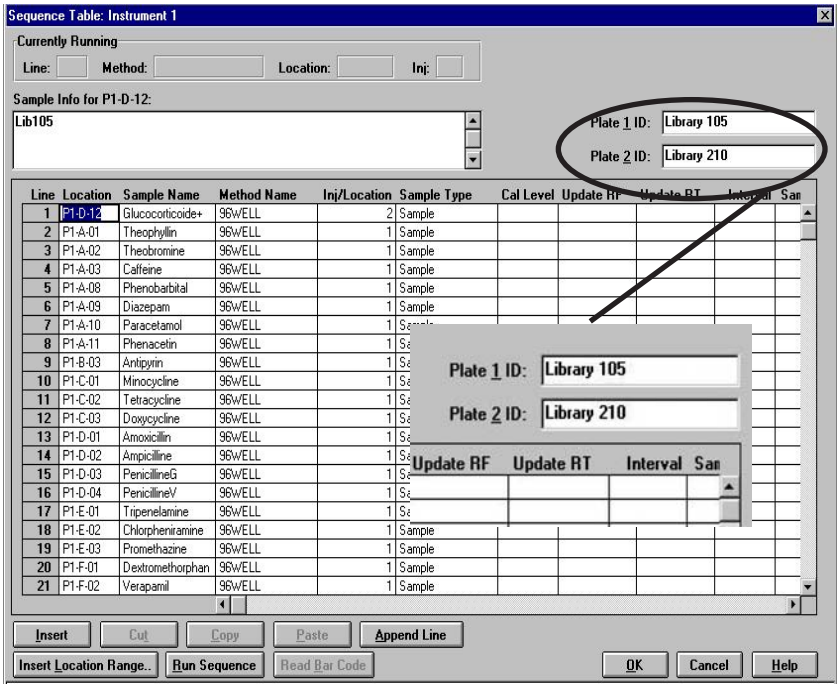


Figure 5
Well-plate name entry

Lowest carry-over

Carry-over is one of the severe factors that can falsify quantitative and qualitative data in HPLC. Today detectors are more and more sensitive and quantitation in the low picogram range is achieved using highly sensitive MS methods. Where samples of very high (mg/ml) and very low (ng/ml) concentration are analyzed in a row, carry-over becomes a critical parameter using these highly sensitive detectors.

In figure 6 a comparison is made between carry-over for an injection with standard wash and an injection using an injector program with additional injection valve switching. The compound used was beclomethasone and 1000 ng were injected followed by the injection of 5 µl methanol. Beclomethasone was selected because carry-over is rather critical for this compound. With standard injection the carry-over was found to be 0.03 %. Using the injector program the carry-over was down to 0.003 %.

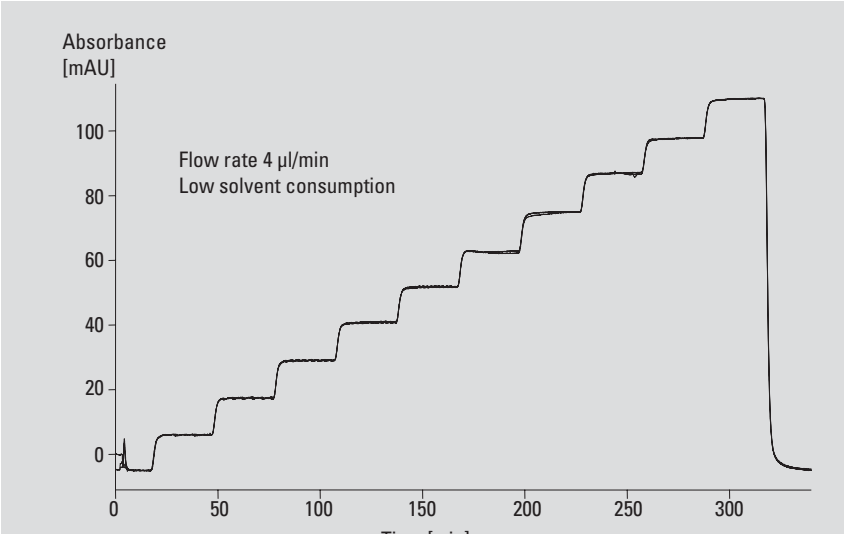


Figure 6
Optimization for lowest carry-over using an injector program

Column:	2.1 × 30 mm Zorbax Eclipse XDB-C18, 3.5 µm
Mobile phase:	water/acetonitrile
Flow rate:	0.2 ml/min
Gradient:	at 0 min 30 % ACN at 2.5 min 30 % ACN at 3 min 80 % ACN at 4 min 30 % ACN at 9 min 30 % ACN
Injection:	0.5 µl for sample, 5 µl for methanol injection
Outside wash of needle before injection:	14 s with methanol using flush port
Column temp.:	40 °C
UV detector:	DAD 254/20 nm reference 350/80 nm

Injector program:
Draw 0.5 (5) µl from sample
Needle wash as method
Inject
Wait 7 minutes
Valve bypass
Wait 0.2 minutes
Valve mainpass
Valve bypass
Valve mainpass

Recommendations for lowest carry-over

The carry-over experiments showed that for standard injection with needle wash in flush port carry-over is typically below 0.01 %. Further reduction of carry-over can be obtained using an injector program with additional injection valve switching. A disadvantage of this method is that overlapped injection is not possible together with this injector program. The injection type needed depends on the injected sample concentrations and on the chemical structure of the molecules. To provide lowest possible carry-over the following is recommended.

- Prime flush pump daily for three minutes with appropriate solvent previous to the first run.
- Set needle wash in flush port to at least10 seconds.
- Use previously described injector program as injection mode if carry-over is significantly higher than 0.01 %.
- For samples where needle outside cannot be cleaned sufficiently with water or alcohol, use wash vials with an appropriate solvent. With an injector program several wash vials can be used for cleaning.

If the needle seat is contaminated and carry-over is significantly higher than expected, the following procedure can be followed to clean the needle seat:

- Go to *More Injector* and set needle to home position.
- Pipette an appropriate solvent onto the needle seat. The solvent should be able to dissolve the contamination. If this is not known use two or three solvents of different polarity. Use several milliliters to clean the seat. The liquid leaves the seat via the drainage for the flush port.
- Clean the needle seat with a tissue and remove all liquid from it.
- Reset the injector.

Fast injection cycles

Short injection cycle times for high sample throughput is one of the main issues in an analytical laboratory today. Shortening cycle time starts with shortening column length, increasing flow rates and steepening gradients. Having optimized these parameters the speed of the autosampler can become a limiting factor where drawing and ejecting a sample takes about two to three minutes and/or overlapped injection is not possible.

The Agilent 1100 Series well-plate sampler described here is able to draw and inject a sample within 36 seconds, including an outside needle wash of 10 seconds.

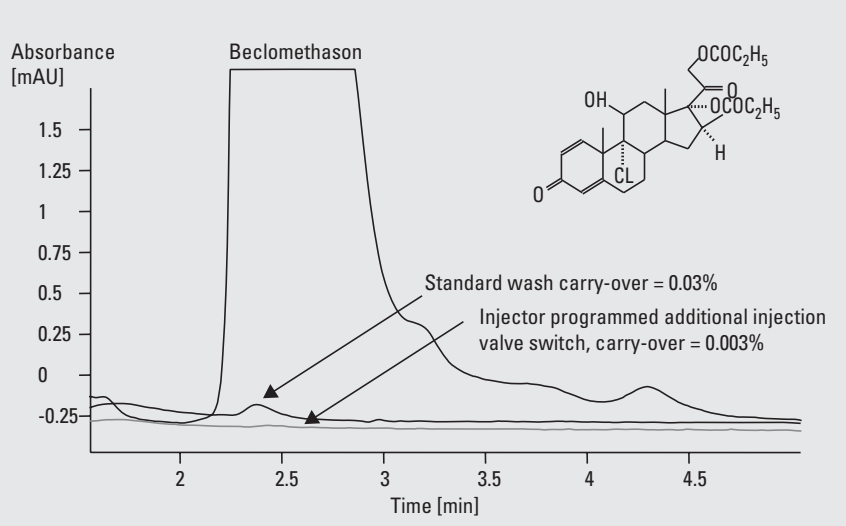


Figure 7
Ultra fast sequence with overlapped injections

Column:	4.6 × 30 mm Zorbax SB-C-18, 3.5 µm
Mobile phase:	water/acetonitrile 60/40
Flow rate:	3 ml/min
Gradient:	at 0 min 40 % ACN
	at 0.3 min 40 % ACN
	at 0.6 min 55 % ACN
	at 0.7 min 40 % ACN
Injection:	1 µl for sample
Outside wash of needle before injection:	10 s with methanol using flush port

Overlapped injection after sample flushed on column (switching time calculated by the instrument based on flow rate and injection volume)

Column temperature:	40 °C
UV detector:	DAD 254/20 nm, reference 350/80 nm

Further reduction of cycle times can be obtained using the overlapped injection mode described previously. In figure 7 the analysis of antipyrine, phenacetin and diazepam is shown using overlapped injections. For this sequence detector balancing performed before each run was switched off. Experiments showed that for well-equilibrated systems no reduction in performance occurs. The cycle time is now down to 1 minute and 6 seconds. This means 54 runs can be completed in 1 hour.

For the last injection of the sequence with overlapped injections it has to be considered that for this run the injection valve is not switched as for the previous runs and consequently the injector delay volume is not bypassed. This means the retention times are prolonged for the last run. Especially at low flow rates this can lead to retention time shifts. To overcome this it is recommended to add an additional "blank" injection as last injection to the sequence.

Recommendations for fast cycle times

The first step to provide short cycle times is to optimize the chromatographic conditions. The autosampler parameters should be set to:

- overlapped injection mode
- wash time for needle outside: 10 seconds
- detector balance: off
- increase of draw and eject speed for large injection volumes
- at last run add a blank, if overlapped injection is used.

Optimum precision of areas

Injection volumes depend on the concentration of samples. The wider the injection volume range of an autosampler the better for different sample concentrations. For a well-plate sampler it is important to show good precision over the complete injection volume range. The precision for different injection volumes was measured for 0.5, 1, 3, 5, 10, 25, 50 and 100 µl injection volume. Results are shown in table 1.

Recommendation for highest precision

For optimum area precision the injection volume should be at least 1 µl. The draw and eject speed should be lowered if sample viscosity is increased. For high injection volumes greater than 50 µl the draw speed should be lower than the default value.

Injection volume (µl)	%RSD of areas for antipyrine	%RSD of areas for phenacetin	%RSD of areas for diazepam	Areas counts mean value
0.5	1.54	1.44	1.90	220
1	0.50	0.49	0.48	200
3	0.17	0.19	0.22	500
5	0.13	0.13	0.13	1000
10	0.23	0.20	0.21	450
25	0.33	0.31	0.33	1200
50	0.02	0.04	0.16	700
100	0.02	0.03	0.07	5000

Table 1
Precision for different injection volumes

Linearity of injection volume

Good linearity in the low and in the high micro-liter injection volume range is another demand that should be fulfilled by a well-plate sampler. Figure 8 shows the volume linearity over an injection volume range from 0.1 up to 10 µl. The system is linear from 0.5 µl. Below 0.5 µl injection volume linearity is not given. The injection was done using a 300-µl well-plate and as sample compound diazepam was chosen. The coefficient of correlation is greater than 0.9999 from 0.5 up to 10 µl.

Figure 9 shows the linearity of injection volume from 10 to 100 µl. Here linearity is given over the complete range. 1.5-ml vials were used for injections and diazepam was used as sample compound.

Recommendations for optimum linearity

In order to provide best possible linearity the injection volume should be at least 0.5 µl. The injector is then typically linear up to 100 µl.

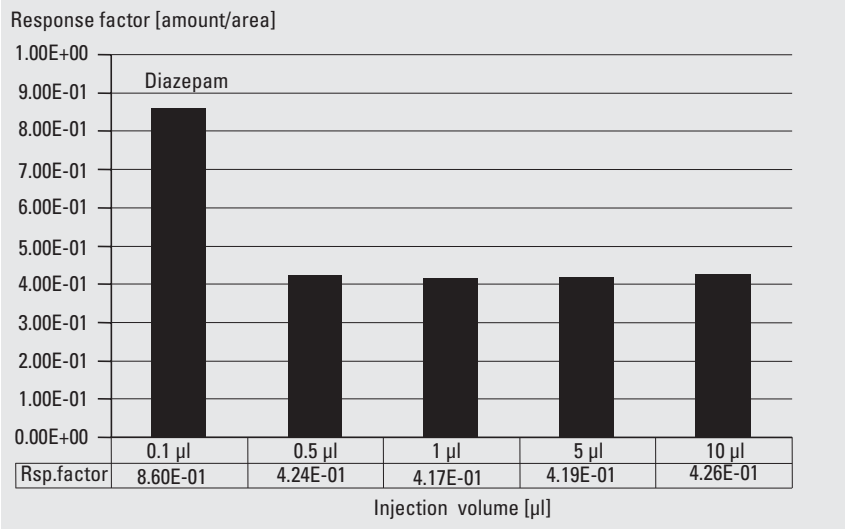


Figure 8
Linearity of injection volume in the low micro-liter range, injected from 300-µl well-plate

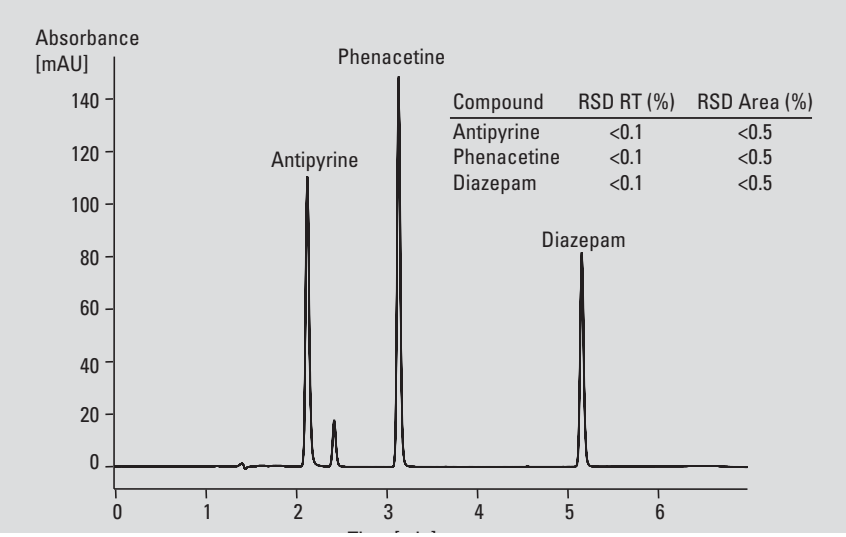


Figure 9
Linearity of injection volume in the middle and high micro-liter range, injected from 1.5 -ml vial

Conclusion

The Agilent 1100 Series well-plate sampler shows optimum performance if some rules for set up are followed. These include using the appropriate solvent for washing the needle outside, using overlapped injections for fast analysis cycle times and injecting more than 1 µl to achieve highly precise quantitative data. It was proven that in general carry-over is below 0.01 % using the standard wash procedure and below 0.005 % carry-over using an injector program with additional steps. Cycle time for a gradient analysis with high flow rate and a short column can be as low as 1 to 2 minutes taking advantage of the fast injection speed of the autosampler and the overlapped injection tool. Linear injection from 0.5 up to 100 µl can be expected as well as high precision for area quantitation from 1 to 100 µl.

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