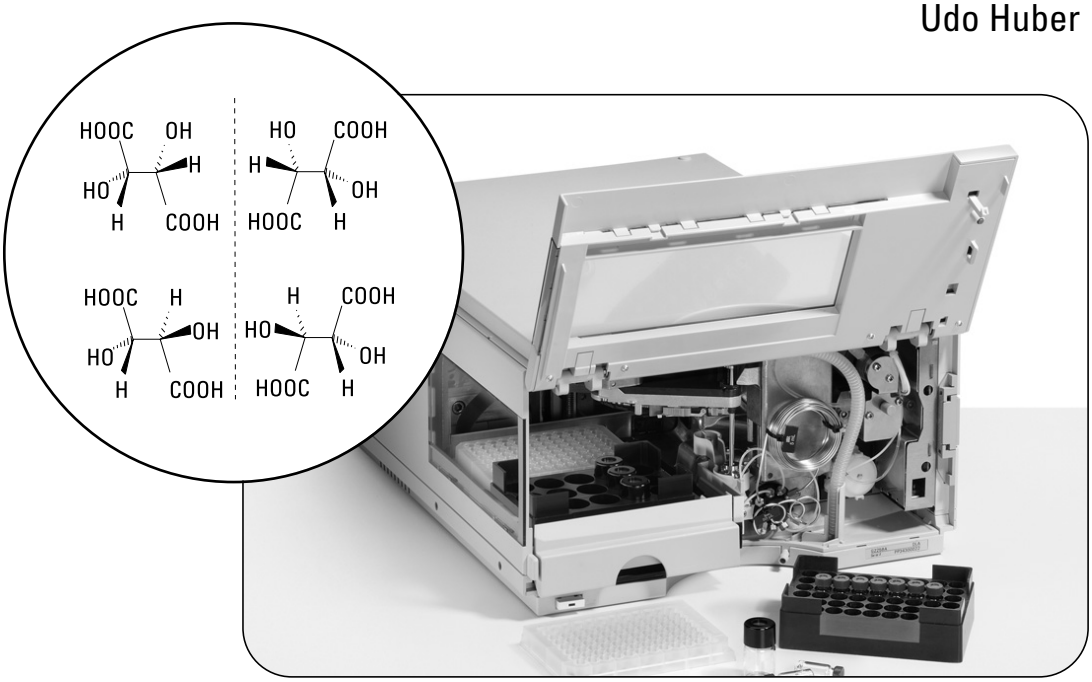
Performing multiple injections in an isocratic purification experiment using the Agilent 1100 Series purification system

Application Note

Udo Huber



Abstract

Separation and purification of stereoisomers, diastereomers and enatiomers are usually performed in relatively long, consecutive, isocratic runs. This Application Note shows how the run time, and therefore the mobile phase consumption, can be lowered by injecting the same sample multiple times within the same purification run. With the Agilent 1100 Series dual-loop autosampler, an integral part of the Agilent 1100 Series purification system[1,2] this can be easily achieved by setting up an injector program. In this Application Note we will explain the commands used in this injector program and show the separation of two steroid isomers as an application example.

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Introduction

Preparative HPLC is currently the method of choice for the purification of compounds in drug discovery. Large numbers of crude samples are purified using generic methods with short and steep gradients of about 10 minutes. While most compounds can be isolated using these methods, the isolation and purification of stereoisomers or diastereomers is much more challenging. The separation of these compounds usually requires much longer run times, either under isocratic conditions or with very shallow gradients. The separation of enantiomers on chiral stationary phases is also performed isocratically, very often with run times of one or two hours.

In this Application Note we will demonstrate how sample throughput can be increased by injecting the sample several times within one run, saving precious time and mobile phase. This can be achieved by setting up an injector program for the Agilent 1100 Series dual-loop autosampler. An application example for the separation of two steroid isomers is also shown.

Equipment

The experiments were performed on an Agilent 1100 Series purification system containing the following modules:

- Two Agilent 1100 Series preparative pumps
- Agilent 1100 Series dual-loop autosampler PS
- Agilent 1100 Series column organizer
- Agilent 1100 Series multi-wavelength detector
- Agilent 1100 Series fraction collector PS

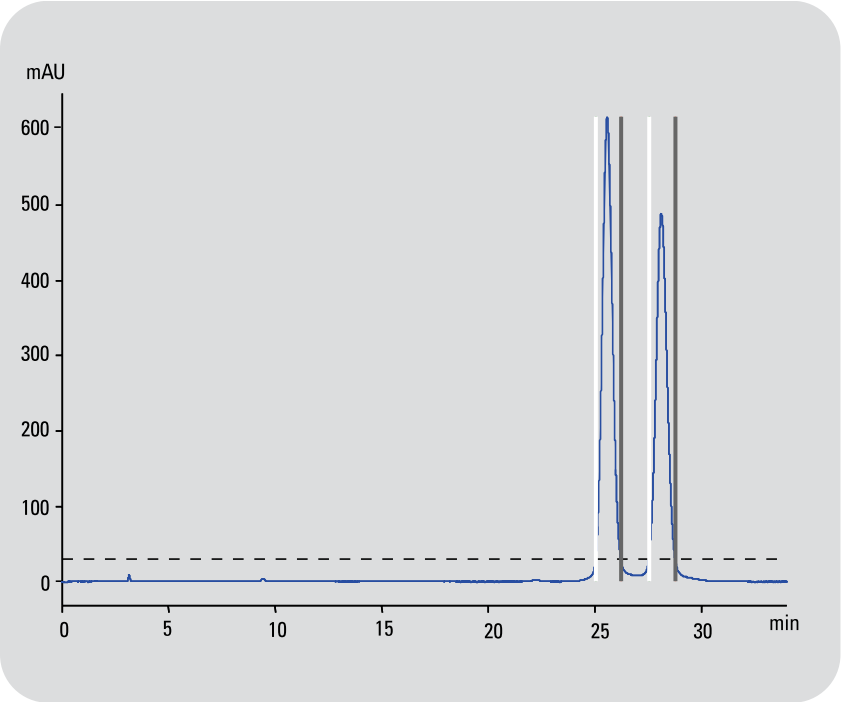
The system was controlled using the Agilent ChemStation (rev. B.01.01).

Results and discussion

Single injection

Subsequent to method optimization the two steroid isomers could be baseline-separated in an isocratic run on a Zorbax XDB C-18 column of 250 mm length. The run time was 35 minuters using a mobile phase of water and acetonitrile (65:35 v/v) as shown in figure 1. The mobile phase consumption was 735 mL per sample injection using a flow rate of 21 mL/min. Fractions were collected based on threshold with a threshold setting of 30 mAU.

For example, solvent consumption would be 3675 mL with an overall run time of 175 minutes for five consecutive injections.



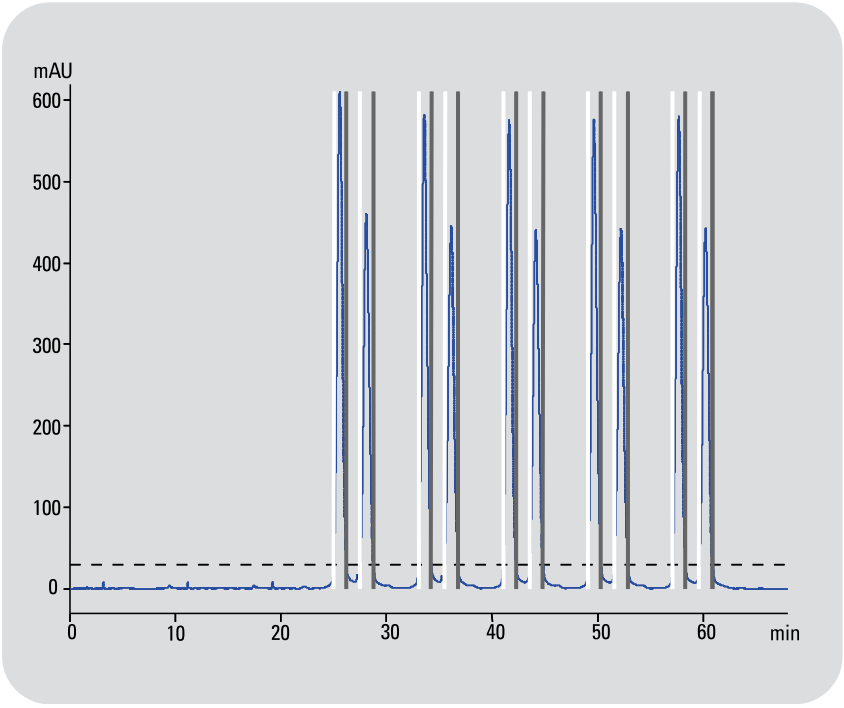
Column:	ZORBAX XDB C-18 21.2 x 250 mm, 7 µm
Mobile phases:	Water = A Acetonitrile = B
Isocratic:	at 35 % B
Stop time:	35 min
Flow:	21 mL/min
Injection:	500 µL
Column temp.:	ambient
UV detector:	DAD 245 nm/8 (ref. 360 nm/50) Prep. flow cell (3-mm pathlength)

Figure 1
Single injection for the separation of two steroid isomeres

Multiple injections

It is possible to inject additional sample onto the column after a certain time as the first isomere elutes after about 25 minutes and there are no other impurities in the sample. This duration is determined by the time difference between the start of the first peak and the end of the second peak, which is approximately 7 minutes. The result for five consecutive injections is shown in figure 2.

The overall run time for five injections was 70 minutes with a mobile phase consumption of 1470 mL. Compared to five single injections (175 minutes, 3675 mL), this equals a time and mobile phase saving of about 60 %.



Column:	ZORBAX XDB C-18 21.2 x 250 mm, 7 µm
Mobile phases:	Water = A Acetonitrile = B
Isocratic:	at 35 % B
Stop time:	70 min
Flow:	21 mL/min
Injection:	5 × 500 µL
Column temp.:	ambient
UV detector:	DAD 245 nm/8 (ref. 360 nm/50) Prep. flow cell (3-mm pathlength)

Figure 2
Multiple injections

Injector program

The injector program for multiple consists of 10 lines, which include washing the needle's surface and flushing the needle, needle seat, needle seat capillary and valve after ejecting the sample into the sample loop. Copies of these ten commands have to be made, depending on the number of injections done in a run. A screen-shot of the injector program is shown in figure 3, the commands are explained in table 1.

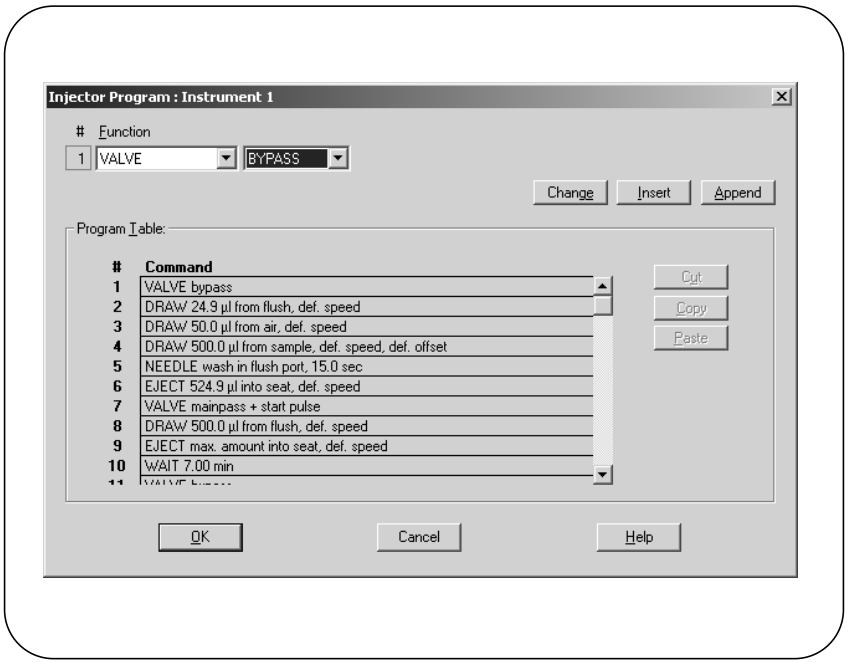


Figure 3
Injector program in the Agilent ChemStation

1	VALVE bypass	Switches sample loop out of flow path from pump to column.
2	DRAW 24.9 µL from flush, def. speed	Seat capillary volume (20.0 µL for lower seat, 24.0 µL for upper seat) and valve volume (4.9 µL) drawn from flush.
3	DRAW 50.0 µL from air, def. speed	Draws air gap between rinse solvent and sample, which is required for best recovery results.
4	DRAW 500 µL from sample, def. speed, def. offset	Sample volume drawn into needle and buffer loop. 500 µL in this application.
5	NEEDLE wash in flush port, 15.0 sec.	Washes needle surface in flush port.
6	EJECT 524.9 µl into seat, def. speed	Ejection of sample into sample loop (eject vol.= sample vol. + seat vol. + valve vol., air is not ejected into loop).
7	VALVE mainpass + start pulse	Switches sample loop into flow path from pump to column and transmits start pulse. For subsequent injections the *VALVE mainpass* command without start pulse must be used.
8	DRAW 500 µL from flush, def. speed	Draws 500 µL rinse solvent.
9	EJECT max. amount into seat, def. speed	Flushes needle seat, needle seat capillary and valve. Empties syringe entirely.
10	WAIT 7.00 min	Wait time prior to subsequent injection.

Table 1
Commands used in injector program

Limits and restrictions

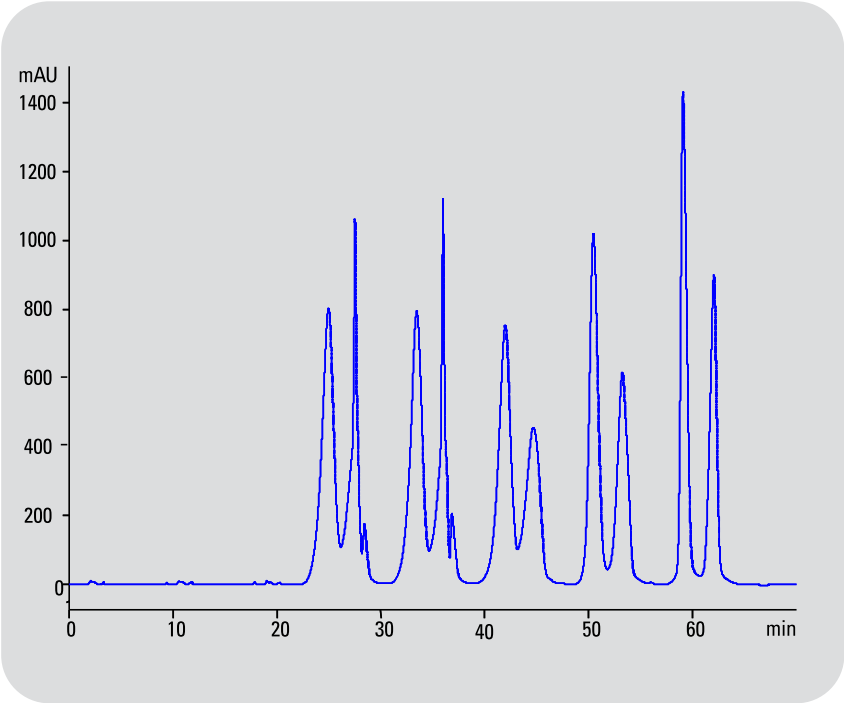
- The mobile phase must be used as the sample solvent. If strong solvents like DMSO, for example, are used the increasing amount of sample solvent influences the chromatography as shown in figure 4.

- The sample loop used for multiple injections has to be set up in the *Set up Injector* window under *Use Loop*.

- The number of lines in the injector program is limited to 60 steps. The number of steps can be increased to approximately 120 by using the command ExtInjProg=1 in the USER.MAC under the \CHEM32\CORE directory. In doing so the method can no longer be used with the hand-held controller.

- To lower the number of injector program lines the REPEAT/END REPEAT command can be used. Please refer to the ChemStation online help.

- Automated pooling of fractions is not available within a single run. Fractions must be pooled manually after the run is completed.



Column:	ZORBAX XDB C-18 21.2 x 250 mm, 7 µm
Mobile phases:	Water = A Acetonitrile = B
Isocratic:	at 35 % B
Stop time:	70 min
Flow:	21 mL/min
Injection:	5 × 500 µL (injector program)
Sample solvent:	DMSO
Column temp.:	ambient
UV detector:	DAD 245 nm/8 (ref. 360 nm/50) Prep. flow cell (3-mm pathlength)

Figure 4
Injection with DMSO as the sample solvent

Conclusion

Performing multiple injections in a single isocratic run can save precious time and mobile phase for the separation and purification of isomers or enatiomers. With the Agilent 1100 Series purification system, multiple injections can be easily performed by setting up an injector program.

References

1.
"Agilent 1100 Series purification system", *Agilent Technologies Brochure*, **2004**, publication number 5989-1255EN.

2.
"Agilent 1100 Series Purification Platform - Customized system configurations for highest purity and recovery", *Agilent Technologies Configuration Guide*, **2004**, publication number 5989-0648EN.

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