Near zero carryover performance of the Agilent 1200 Series HiP Autosampler SL Plus

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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 could 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

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, needle seat cleaning and fittings. Further, a new treatment was developed for all flow capillaries to passivate any active sites on the inner surfaces. In this poster the performance is described relating to carryover, injection volume linearity, area precision

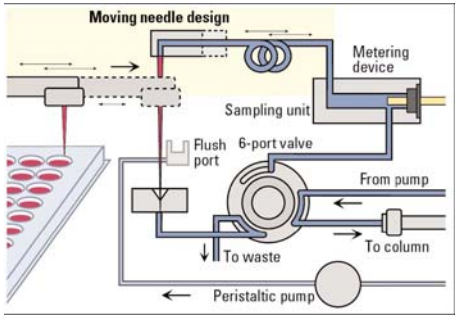
Experimental

- 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 um columns

Results and Discussion

The Agilent 1200 Series SL Plus autosampler (ALS) is based on the flow through design. The flow through design offers a wide linear injection volume range and the possibility to inject small sample volumes without loss of sample. During the injection routine of the ALS 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. 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. 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 ALS and prevents contamination of the needle seat. The flush port of the ALS is refilled with fresh solvent by a peristaltic pump that is installed in the ALS 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.

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



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

Carryover behavior

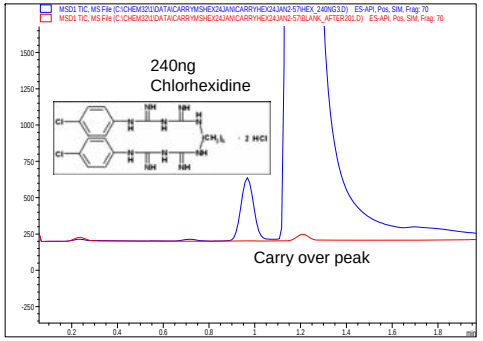
The carryover behavior of the Agilent 1200 Series high performance autosampler SL Plus was evaluated using three different compounds. Chlorhexidine and beclomethsone are well known to cause severe carry over when injected at high concentration. The third compound, primidone, is less critical. To evaluate carryover, the Agilent 6140 MS was used in selected ion mode (SIM).

The target mass for Chlorhexidine 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 compound. 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.

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

Overlay of 240 ng Chlorhexidine and blank injections.

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



Carryover behavior of Beclomethason

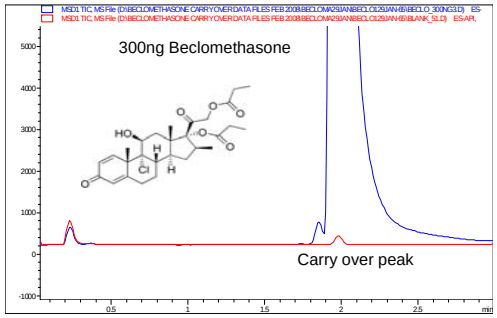
Beclomethasone is a synthetic corticosteroid and has anti-inflammatory properties. Beclomethasone dipropionate is a pro-drug to beclomethasone and was used as test compound because it is also known to cause severe carryover. It dissolves well in Methanol.

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,. 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.

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

Overlay of highly concentrated sample with blank run.

Carryover was found to be about 0.0022 %, equivalent to about 6.5 pg,



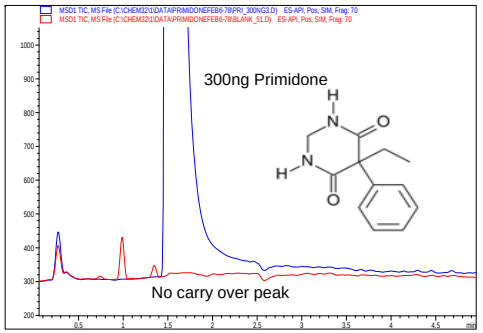
Carryover behavior of Primidone

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.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,. 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.

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

Overlay of high concentration and blank run

The carryover was found to be less than the LOD.



Summary

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.

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.