Comparison of the Agilent 1260 Infinity Autosampler with the Standard Autosampler

Demonstration of Backwards Compatibility by Analysis of Antiepileptic Compounds

Technical Overview

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Abstract

For laboratories running routine analyses that require a reliable system with UHPLC efficiency, the Agilent 1260 Infinity LC is the instrument of choice. With the introduction of the Agilent 1260 Infinity Autosampler, the flexibility and efficiency of the system are raised to a higher level. Despite newly introduced and improved features, critical instrument parameters of the 1260 Infinity Autosampler and its predecessor are equivalent, which facilitates method transfer and minimizes instrument revalidation in regulated environments. This Technical Overview describes a comparison of the 1260 Infinity Autosampler with its predecessor, the Agilent 1260 Infinity Standard Autosampler. Analyses of six antiepileptic compounds were evaluated regarding retention time and area precision, resolution, linearity, and sensitivity. The results show that the 1260 Infinity Autosampler performs equivalently to the 1260 Infinity Standard Autosampler. However, the 1260 Infinity Autosampler provides advantages in versatility and the potential reduction in height of the system, since the column compartment and sample cooling can be integrated.

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Introduction

The Agilent 1260 Infinity LC is a robust and versatile system. With an extended pressure range up to 600 bar, the system delivers true UHPLC performance and high resolution, making it a preferred choice for pharmaceutical method development and quality control (QC) analyses. The Agilent 1260 Infinity Autosampler adds features that make the 1260 Infinity LC even more flexible, while remaining compact and affordable[1]:

- Extended capacity (>30 %) for up to 132 vials (2 mL) or 36 vials (6 mL)
- Injection cycle sped up by more than 30 seconds[1,2]
- Needle flush port for outside rinsing of the needle to maintain lowest carryover (<40 ppm)
- Integrated sample cooling (optional) down to 4 °C
- Integrated column compartment (optional) holding two columns up to 30 cm length, providing heating capacity up to 80 °C

These benefits make the 1260 Infinity Autosampler highly attractive for analytical laboratories. Instrument qualification and system suitability tests might be required, however, for GxP regulated environments. This Technical Overview compares the performance of three different system configurations featuring the Agilent 1260 Infinity Standard Autosampler and the 1260 Infinity Autosampler. The latter was evaluated both with and without the optional integrated column compartment. A mixture of six common antiepileptic standard compounds served as an example, representing a typical sample of a pharmaceutical method development or quality control (QC) laboratory. The systems were evaluated regarding retention time (RT) and area precision, resolution, linearity, and sensitivity.

Experimental

Instrumentation

The Agilent 1260 Infinity LC with the Agilent 1260 Infinity Standard Autosampler comprised the following modules:

- Agilent 1260 Infinity Binary Pump (G1312B), equipped with a solvent selection valve (Option 60068)
- Agilent 1260 Infinity Standard Degasser (G1322A)
- Agilent 1260 Infinity Standard Autosampler (G1329B)
- Agilent 1290 Infinity Thermostat (G1330B)
- Agilent 1260 Infinity Thermostatted Column Compartment (G1316A)
- Agilent 1260 Infinity Diode Array Detector (G4212B), equipped with a 10 mm Max-Light cartridge cell (1.0 µL)

The Agilent 1260 Infinity LC system used for comparison of the 1260 Infinity Autosampler comprised the following modules:

- Agilent 1260 Infinity Binary Pump (G1312B), equipped with a solvent selection valve (Option 60068)
- Agilent 1260 Infinity Standard Degasser (G1322A)
- Agilent 1260 Infinity Autosampler (G7129A) with sample cooler (Option #100)
- Agilent 1260 Infinity Thermostatted Column Compartment (G1316A)
- Agilent 1260 Infinity Diode Array Detector (G4212B), equipped with a 10 mm Max-Light cartridge cell (1.0 µL)

The third system configuration comprised the same modules as the second, except for the Agilent 1260 Infinity Thermostatted Column Compartment (G1316A), which was replaced by an integrated column compartment with a 3 µL heater (G7130-60030).

Column

Agilent ZORBAX SB-C18,
4.6 × 150 mm, 5 µm (p/n 883975-902)

Software

Agilent OpenLAB CDS ChemStation Edition for LC and LC/MS Systems, version C.01.07 SR1 [106]

Solvents

Solvent A

Water (fresh ultrapure water obtained from a Milli-Q integral system equipped with a 0.22 µm membrane point-of-use cartridge (Millipak)

Solvent B

Acetonitrile (LC grade)

Samples

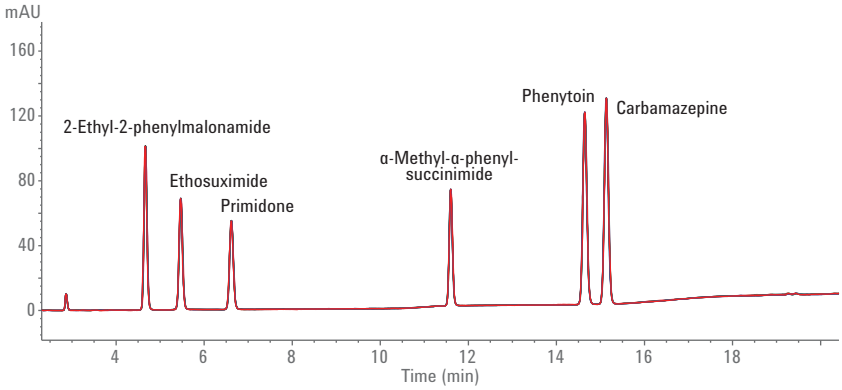
A mix of six typically used antiepileptic compounds (2-ethyl-2-phenylmalonamide, ethosuximide, primidone, α-methyl-α-phenyl succinimide, phenytoin, and carbamazepine) was analyzed[3,4]. Eight calibration points at 200, 100, 50, 25, 12.5, 6.25, 3.125, and 0.781 ng/µL were prepared and injected three times each in random order. Calibration curves were constructed using linear regression. Limits of detection (LOD) and quantification (LOQ) were calculated for each compound based on a signal-to-noise ratio (S/N) greater than 3 and 10, respectively, extrapolated from the lowest concentrated calibration point. For determination of retention time relative standard deviation (RT RSD), area RSD, and resolution, 10 consecutive runs of the 25 ng/µL mix were used. Analyses were conducted on all three instrument configurations described in the Instrumentation section.

Chromatographic conditions

Parameter	Value
HPLC Column	4.6 × 150 mm, 5 µm
Mobile phase	A) Water B) Acetonitrile
Flow rate	0.8 mL/min
Gradient	0 minutes–15 %B 8 minutes–22 %B 9 minutes–30 %B 13 minutes–35 %B 17 minutes–70 %B 20 minutes–95 %B
Stop time	25 minutes
Post time	15 minutes
Injection volume	5 µL
Column temperature	60 °C
Sample temperature	8 °C
Detection	Signal A 204/4 nm, reference 360/80 nm Peak width > 0.013 minutes (0.25 seconds response time)

Results and Discussion

A standard mix of six antiepileptic compounds was analyzed using a 1260 Infinity LC equipped with a 1260 Infinity Standard Autosampler. Ten consecutive runs were evaluated for RT and area precision, as well as for resolution (Figure 1). Both RT and area precision were well within specifications; RT RSDs were below 0.06 %, and area RSDs were below 0.12 % for all compounds except ethosuximide, which was below 0.23 %.

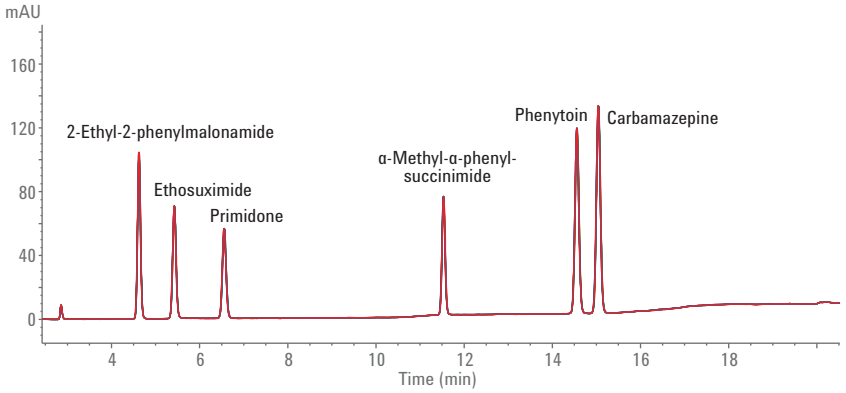


Analyte	RT (min)	RT RSD (%)	Area RSD (%)	Resolution
2-Ethyl-2-phenylmalonamide	4.67	0.060	0.090	—
Ethosuximide	5.47	0.047	0.223	5.9
Primidone	6.62	0.041	0.113	7.5
α-Methyl-α-phenyl succinimide	11.60	0.018	0.089	33.2
Phenytoin	14.64	0.016	0.093	19.6
Carbamazepine	15.13	0.017	0.090	2.8

Figure 1. HPLC analysis of six antiepileptic compounds (25 ng/µL) analyzed on an Agilent 1260 Infinity LC equipped with an Agilent 1260 Infinity Standard Autosampler and an Agilent 1260 Infinity Thermostatted Column Compartment. Overlay of 10 consecutive runs and data on RT and area precision, and resolution.

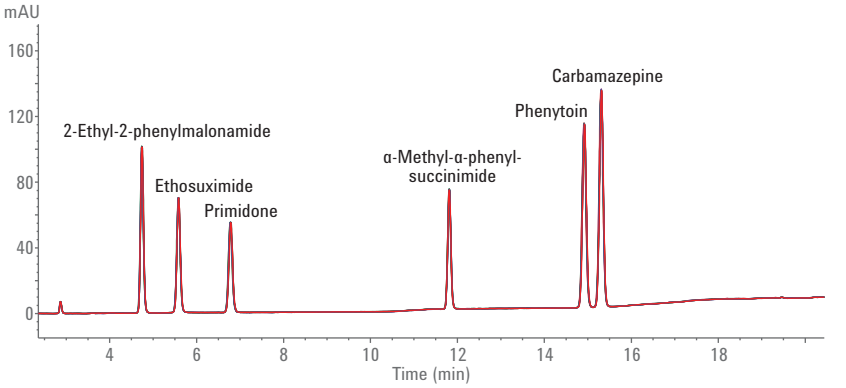
The standard mix was also analyzed on a 1260 Infinity LC equipped with a 1260 Infinity Autosampler. Ten consecutive runs were evaluated for RT and area precision, as well as for resolution (Figure 2). RTs of all six compounds could be reproduced within a margin of less than 1 %. RSDs of RT and area were comparable to those of the first system configuration, and below 0.06 and 0.23 %, respectively. Resolution was equivalent to the first system configuration.

In the third system configuration, an integrated column compartment located within the 1260 Infinity Autosampler replaced the 1260 Infinity Thermostatted Column Compartment. Again, 10 consecutive runs of the standard mix were evaluated for RT and area precision, and resolution (Figure 3). Compared with the first system configuration featuring a 1260 Infinity Standard Autosampler, RTs were slightly higher (1.2 to 2.4 %). RT RSDs, however, were equivalent to the other system configurations, and were well below 0.07 %. Area RSDs were comparable to the first and second system configurations, and did not exceed 0.20 %. Resolutions of the six compounds did not change significantly relative to the previous system configurations.



Analyte	RT (min)	RT RSD (%)	Area RSD (%)	Resolution
2-Ethyl-2-phenylmalonamide	4.63	0.042	0.211	—
Ethosuximide	5.43	0.039	0.207	6.0
Primidone	6.56	0.053	0.215	7.5
α-Methyl-α-phenyl succinimide	11.53	0.022	0.228	34.0
Phenytoin	14.56	0.021	0.198	20.0
Carbamazepine	15.05	0.022	0.211	2.9

Figure 2. HPLC analysis of six antiepileptic compounds (25 ng/µL) analyzed on an Agilent 1260 Infinity LC equipped with an Agilent 1260 Infinity Autosampler and an Agilent 1260 Infinity Thermostatted Column Compartment. Overlay of 10 consecutive runs and data on RT and area precision, and resolution.



Analyte	RT (min)	RT RSD (%)	Area RSD (%)	Resolution
2-Ethyl-2-phenylmalonamide	4.74	0.059	0.097	—
Ethosuximide	5.58	0.054	0.199	6.2
Primidone	6.78	0.065	0.129	7.8
α-Methyl-α-phenyl succinimide	11.81	0.026	0.107	33.7
Phenytoin	14.92	0.028	0.134	20.1
Carbamazepine	15.31	0.024	0.107	2.3

Figure 3. HPLC analysis of six antiepileptic compounds (25 ng/µL) analyzed on an Agilent 1260 Infinity LC equipped with an Agilent 1260 Infinity Autosampler with an integrated column compartment. Overlay of 10 consecutive runs and data on RT and area precision, and resolution.

On all three system configurations, a calibration with the standard mix was carried out using eight calibration points between 0.8 and 200 ng/µL (Figure 4). LODs and LOQs were calculated based on an S/N greater than 3 and 10, respectively. In the configuration comprising a 1260 Infinity Standard Autosampler and a 1260 Infinity Thermostatted Column Compartment, the correlation coefficients of all six calibration curves were greater than 0.9991. The LOD and LOQ were below 2 and 6 ng, respectively (Table 1).

The calibration was also carried out on the system configuration featuring the 1260 Infinity Autosampler, using the same eight calibration points in triplicate. LODs and LOQs were comparable to those yielded with the first system configuration and even slightly lower for α-methyl-α-phenyl succinimide and phenytoin (Table 1). Correlation coefficients of the linear calibration curves were greater than 0.9991 for all six compounds.

On the third system configuration, featuring the 1260 Infinity Autosampler with the integrated column compartment, the same calibration was measured as in the previous experiments. Correlation coefficients of the linear calibration curves were comparable to or even higher than in the first and second system configurations, respectively ($R^2 \geq 0.9994$, Table 1). LODs and LOQs were lower than on the previous system setups, except α-methyl-α-phenyl succinimide, which had an LOD and LOQ of 2.6 and 8.5 ng compared to 1.5 and 5.1 ng on the first system.

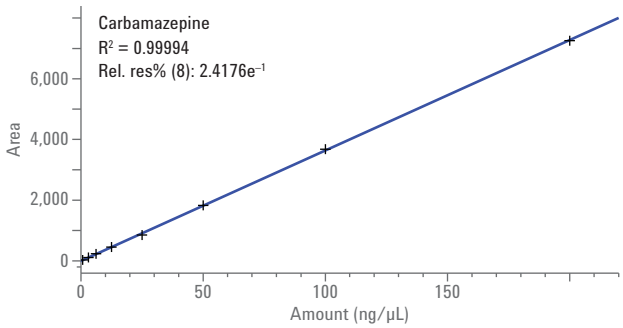


Figure 4. Representative calibration curve (least squares linear regression) of analyte 6 (carbamazepine), analyzed with the first system setup (Agilent 1260 Infinity Standard Autosampler).

Table 1. Calibration data of the six antiepileptic compounds analyzed on three different system setups. Linear calibration curves were constructed using eight calibration points in triplicate from 0.8 to 200 ng/µL. LOD and LOQ are given as absolute amount on column, and were calculated based on an S/N of 3 and 10, respectively.

Analyte	LOD (ng)	LOQ (ng)	Linearity
Configuration with an Agilent 1260 Infinity Standard Autosampler			
2-Ethyl-2-phenylmalonamide	1.2	3.9	0.99990
Ethosuximide	1.6	5.2	0.99918
Primidone	1.8	5.9	0.99993
α-Methyl-α-phenyl succinimide	1.5	5.1	0.99990
Phenytoin	0.7	2.4	0.99986
Carbamazepine	0.9	3.0	0.99994
Configuration with an Agilent 1260 Infinity Autosampler			
2-Ethyl-2-phenylmalonamide	1.2	4.1	0.99965
Ethosuximide	1.6	5.5	0.99919
Primidone	1.9	6.2	0.99962
α-Methyl-α-phenyl succinimide	0.2	0.6	0.99963
Phenytoin	0.1	0.3	0.99964
Carbamazepine	1.1	3.7	0.99961
Configuration with an Agilent 1260 Infinity Autosampler and integrated column compartment			
2-Ethyl-2-phenylmalonamide	0.1	0.2	0.99997
Ethosuximide	0.2	0.5	0.99943
Primidone	0.2	0.6	0.99999
α-Methyl-α-phenyl succinimide	2.6	8.5	0.99996
Phenytoin	0.1	0.3	0.99992
Carbamazepine	0.6	2.0	0.99999

After exchange of the 1260 Infinity Standard Autosampler for the 1260 Infinity Autosampler, a mixture of six antiepileptic compounds could be separated with equivalently high RT and area precision. Using the integrated column compartment of the 1260 Infinity Autosampler instead of the 1260 Infinity Thermostatted Column Compartment did not generate significant changes in RT or area precision. With this system configuration, RTs shifted slightly in absolute terms, but changes were below 2.5 % compared to the 1260 Infinity Standard Autosampler with the 1260 Thermostatted Column Compartment. Linearity, LODs, and LOQs for the compounds analyzed were equivalent, and on an excellent level on all tested system configurations.

Conclusion

This Technical Overview compares the performance of the Agilent 1260 Infinity Autosampler to the Agilent 1260 Infinity Standard Autosampler. The analysis of six antiepileptic compounds was conducted on three different system configurations, two of which featured the 1260 Infinity Autosampler with or without an integrated column compartment. A standard HPLC method was applied and evaluated regarding precision, resolution, sensitivity, and linearity. Results show that the 1260 Infinity Standard Autosampler and the 1260 Infinity Autosampler perform equally well. All three system configurations yielded high linearity, as well as high precision in both area and retention time. Resolution, LODs, and LOQs were on a comparable, excellent level throughout the tested system configurations. The data show that the 1260 Infinity Autosampler can be implemented into existing validated instrument setups with minimum instrument qualification and revalidation procedures.

References

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