Analysis of Pharmaceutical Substances Using HPLC and UHPLC Methods

Backward Compatibility for Validated Analytical Procedures and Methods—Comparing the Agilent 1290 Infinity LC and the Agilent 1290 Infinity II LC

Application Note

Small Molecule Pharmaceuticals

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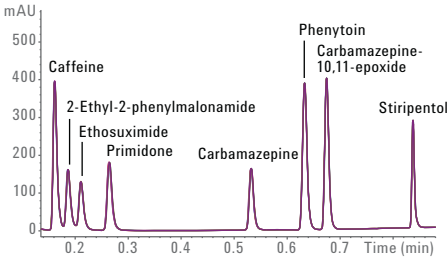
Abstract

For analytical laboratories in GxP regulated environments, the Agilent 1290 Infinity LC has been the instrument of choice for UHPLC, providing excellent confidence and reliability with highest performance. The Agilent 1290 Infinity II LC sets benchmarks in efficiency. The leading UHPLC technology in the 1290 Infinity II LC provides the capacity and speed required for maximum efficiency. This Application Note describes HPLC as well as UHPLC analysis on both systems. We have chosen a selection of antiepileptic drugs as model components to mimic a typical analysis in pharmaceutical development or QC laboratories. The analytical results were compared regarding reproducibility, resolution, linearity, and sensitivity. The performance of the 1290 Infinity II LC can be considered equivalent or better for the analysis of pharmaceuticals, which substantially reduces the effort for method revalidation and method transfer.



Agilent Technologies

Introduction

The Agilent 1290 Infinity LC is not only one of the most powerful, but also the most adaptive UHPLC systems available. The 1290 Infinity LC is able to handle the widest range of applications from legacy HPLC methods to the most complex UHPLC workflows.

The Agilent 1290 Infinity II LC offers expanded capabilities to maximize laboratory efficiency, as well as instrument and analytical efficiency through highest sample capacity and fastest injection cycles, for highest throughput of any application. Especially important in the pharmaceutical industry is the seamless integration in current infrastructures and smooth method transfer from legacy equipment. The main design elements in the 1290 Infinity II LC drive nondisruptive transition to higher productivity and lower costs. The 1290 Infinity II LC provides:

- Lowest dispersion for highest resolution
- Highest peak capacity for challenging separations
- Lowest carryover for highest data quality[1]
- Unique detection capabilities for qualitative as well as quantitative analysis
- Highest retention time precision for reliable peak identification[2]
- High capacity within a small footprint
- Fast injection cycles with dual-needle injection

With design elements such as sample hotel, needle-handling routines, and carryover reduction, the Agilent 1290 Infinity II Multisampler enables the handling of many different samples and analyte types without any interaction difficulties. With a high-power range pump (up to 1,300 bar) and high-frequency UV detection (up to 240 Hz), the 1290 Infinity II LC represents the optimal UHPLC system, providing maximum efficiency to tackle the challenges of qualitative and quantitative analysis of drug products and drug substances.

This Application Note shows a comparison between the performance of the 1290 Infinity LC and the 1290 Infinity II LC for the analysis of antiepileptic drugs as model components to mimic a typical analysis in the pharmaceutical development or QC laboratory. The systems were evaluated regarding reproducibility, resolution, linearity, and sensitivity.

Experimental

Equipment

The Agilent 1290 Infinity LC system consisted of the following modules:

- Agilent 1290 Infinity Binary Pump (G4220A), equipped with 35-µL Jet Weaver mixer
- Agilent 1290 Infinity Autosampler (G4226A)
- Agilent 1290 Infinity Thermostat (G1330B)
- Agilent 1290 Infinity Thermostatted Column Compartment (G1316C)
- Agilent 1290 Infinity Diode Array Detector (G4212A), equipped with a 10-mm Max-Light cartridge cell

The Agilent 1290 Infinity II LC system consisted of the following modules:

- Agilent 1290 Infinity II High-Speed Pump (G7120A)
- Agilent 1290 Infinity II Multisampler (G7167B), equipped with sample cooler (Option #100)
- Agilent 1290 Infinity II Multicolumn Thermostat (G7116B)
- Agilent 1290 Infinity II Diode Array Detector (G7117B) equipped with a 10-mm Max-Light cartridge cell

Columns

- Agilent ZORBAX SB-C18, 4.6 × 150 mm, 5 µm (p/n 883975-902)
- Agilent ZORBAX RRHD SB-C18, 2.1 × 50 mm, 1.8 µm (p/n 857700-902)

Software

Agilent OpenLAB CDS ChemStation Edition for LC and LC/MS Systems, version C.01.07 [27]

Solvents and samples

Solvents

Solvent A = Water
Solvent B = Acetonitrile

Sample

A mix of seven typically used antiepileptic drugs (2-ethyl-2-phenylmalonamide, ethosuximide, primidone, carbamazepine, phenytoin, carbamazepine-10,11-epoxide, and stiripentol, 25 ng/µL each) was used, with caffeine as a reference substance.

All solvents were LC grade. Fresh ultrapure water was obtained from a Milli-Q Integral system equipped with a 0.22-µm membrane point-of-use cartridge (Millipak). The antiepileptic drug standards were purchased from Sigma-Aldrich, St. Louis, Missouri, US.

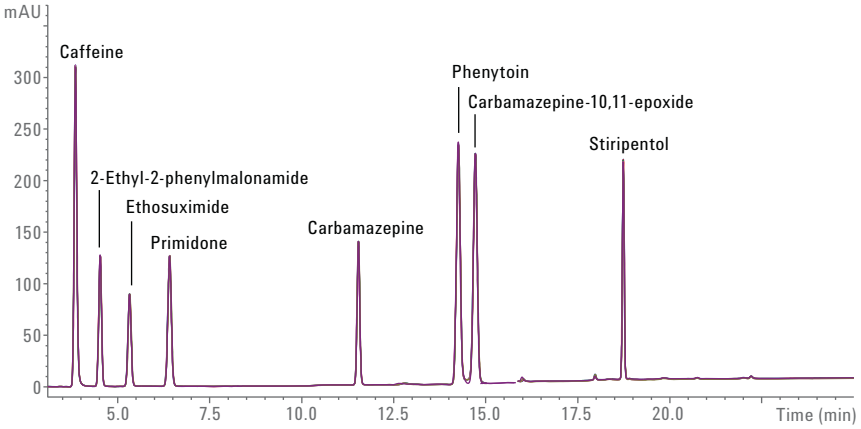
Chromatographic conditions

HPLC with a 4.6 × 150 mm, 5-µm column	
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
Detection	Signal A 204/4 nm, reference 360/80 nm Peak width > 0.025 minutes (0.5-seconds response time) Data rate 10 Hz
UHPLC with 2.1 × 50 mm, 1.8-µm column, simple method transfer	
Mobile phase	A) Water B) Acetonitrile
Flow rate	0.2 mL/min
Gradient	0 minutes – 15 %B 2.67 minutes – 22 %B 3.00 minutes – 30 %B 4.33 minutes – 35 %B 5.67 minutes – 70 %B 6.67 minutes – 95 %B
Stop time	8.50 minutes
Post time	5 minutes
Injection volume	1.25 µL
Column temperature	60 °C
Detection	Signal A 204/4 nm, reference 360/80 nm Peak width > 0.025 minutes (0.5-seconds response time) Data rate 10 Hz
UHPLC with 2.1 × 50 mm, 1.8- µm column, method transfer optimized for speed	
Mobile phase	A) Water B) Acetonitrile
Flow rate	1.5 mL/min
Gradient	0 minutes – 15 %B 0.30 minutes – 22 %B 0.35 minutes – 30 %B 0.5 minutes – 35 %B 0.65 minutes – 70 %B 0.8 minutes – 95 %B
Stop time	1 minute
Post time	1 minute
Injection volume	1.25 µL
Column temperature	80 °C
Detection	Signal A 204/4 nm, reference 360/80 nm Peak width > 0.0031 minutes (0.063-seconds response time) Data rate 80 Hz

Results and Discussion

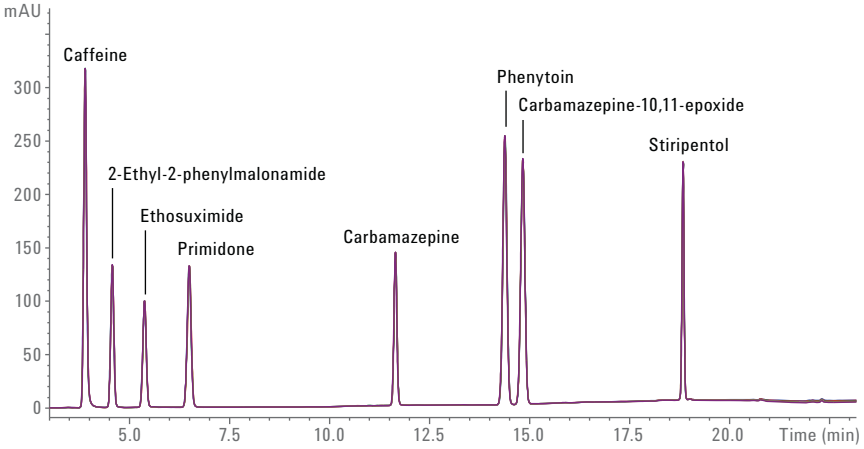
The antiepileptic drug standards were analyzed under HPLC conditions using the 1290 Infinity LC (Figure 1). Six consecutive runs were analyzed for their precision regarding retention time (RT) and area, as well as for resolution. The relative standard deviations (RSDs) of RT and area were found to be excellent, below 0.047 and 0.65 % respectively.

The antiepileptic drug standards were also analyzed under HPLC conditions using the 1290 Infinity II LC (Figure 2). Six consecutive runs were analyzed for their precision regarding RT and area, as well as for resolution. The RSDs of RT and area were also found to be excellent, and even better than the 1290 Infinity LC, below 0.042 and 0.15 % respectively.



	RT RSD (%)	Area RSD (%)	Resolution
Caffeine	0.040	0.153	9.0
2-ethyl-2-phenylmalonamide	0.043	0.083	4.8
Ethosuximide	0.041	0.248	5.2
Primidone	0.046	0.143	6.3
Carbamazepine	0.018	0.203	32.1
Phenytoin	0.019	0.163	16.4
Carbamazepine-10,11-epoxide	0.015	0.648	2.3
Stiripentol	0.007	0.533	7.4

Figure 1. HPLC Analysis of seven antiepileptic drugs and caffeine (overlay of six consecutive runs) on an Agilent ZORBAX SB-C18, 4.6 × 150 mm, 5-µm column, using the Agilent 1290 Infinity LC system.

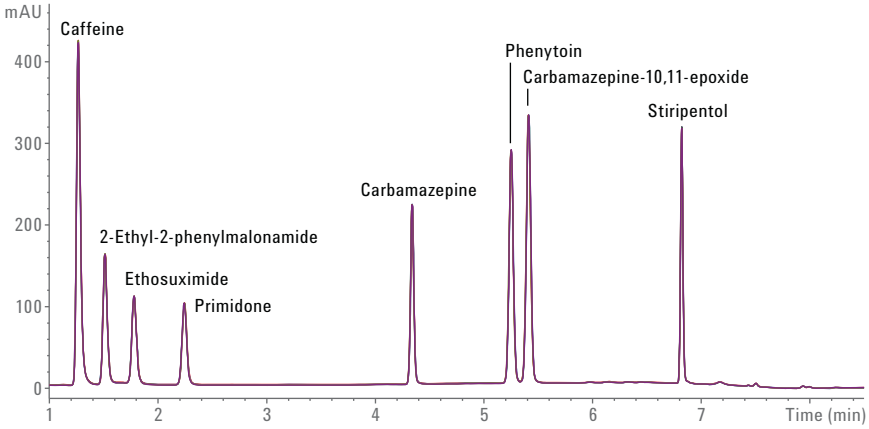


	RT RSD (%)	Area RSD (%)	Resolution
Caffeine	0.035	0.064	4.3
2-ethyl-2-phenylmalonamide	0.039	0.073	4.8
Ethosuximide	0.031	0.111	5.2
Primidone	0.041	0.059	6.4
Carbamazepine	0.015	0.087	32.1
Phenytoin	0.016	0.100	16.5
Carbamazepine-10,11-epoxide	0.008	0.143	2.3
Stiripentol	0.002	0.045	20.8

Figure 2. HPLC analysis of seven antiepileptic drugs and caffeine (overlay of six consecutive runs) on an Agilent ZORBAX SB-C18, 4.6 × 150 mm, 5-µm column, using the Agilent 1290 Infinity II LC system.

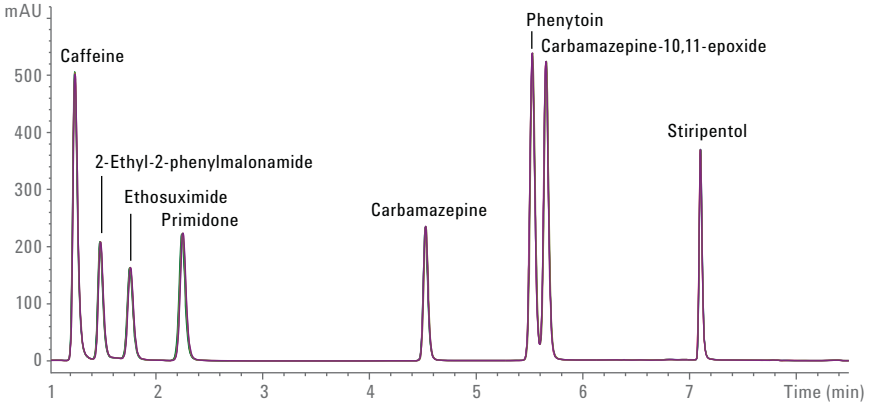
To shorten the analysis time of the drug standards, the method was transferred to an UHPLC method using an Agilent ZORBAX SB-C18, 2.1 × 50 mm, 1.8 µm column. Using simple method transfer without any optimization for speed or resolution, the total cycle time was shortened from 40 to 13.5 minutes, resulting in a total time saving of over 90 % and a solvent saving of over 66 %. Figure 3 shows the overlay of six consecutive runs on the shorter column together with the RSD values for RT and area, as well as the resolution values on the 1290 Infinity LC. The RSDs for RT and area were still found to be good, with values below 0.23 and 0.57 % respectively. Also, the resolution was equivalent to the HPLC method.

Figure 4 shows the overlay of six consecutive runs on the shorter column together with the RSD values for RT and area, as well as the resolution values on the 1290 Infinity II LC. The RSDs for RT and area were found to be slightly better, with values below 0.16 and 0.27 % respectively. The resolution was equivalent.



	RT RSD (%)	Area RSD (%)	Resolution
Caffeine	0.172	0.126	5.6
2-ethyl-2-phenylmalonamide	0.077	0.149	3.0
Ethosuximide	0.163	0.142	3.0
Primidone	0.225	0.120	5.1
Carbamazepine	0.091	0.087	25.4
Phenytoin	0.1	0.291	10.2
Carbamazepine-10,11-epoxide	0.068	0.068	2.2
Stiripentol	0.023	0.560	5.5

Figure 3. UHPLC analysis (simple method conversion) of seven antiepileptic drugs and caffeine (overlay of six consecutive runs) on an Agilent ZORBAX SB-C18, 2.1 × 50 mm, 1.8-µm column, using the Agilent 1290 Infinity LC System.



	RT RSD (%)	Area RSD (%)	Resolution
Caffeine	0.116	0.082	1.6
2-ethyl-2-phenylmalonamide	0.14	0.140	2.8
Ethosuximide	0.134	0.203	3.0
Primidone	0.158	0.091	4.8
Carbamazepine	0.05	0.142	24.4
Phenytoin	0.038	0.145	11.7
Carbamazepine-10,11-epoxide	0.033	0.119	1.5
Stiripentol	0.009	0.264	4.8

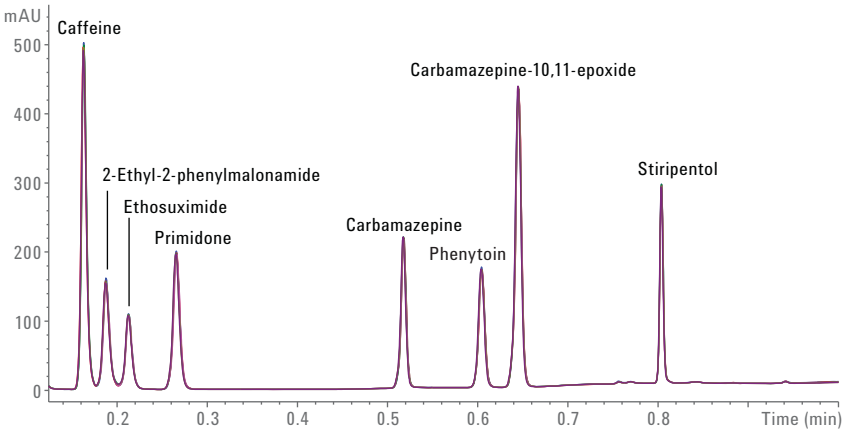
Figure 4. UHPLC analysis (simple method conversion) of seven antiepileptic drugs and caffeine (overlay of six consecutive runs) on an Agilent ZORBAX SB-C18, 2.1 x 50 mm, 1.8-µm column, using the Agilent 1290 Infinity II LC system.

To achieve an ultrafast separation, the UHPLC method was optimized for speed. As a result, a separation within 0.9 minutes was possible with a flow rate of 1.5 mL/min. Figure 5 shows an overlay of six consecutive runs on the 2.1-mm column together with the values for retention time and area precision, as well as resolution on the 1290 Infinity LC.

The RSDs of RTs were found to be below 0.33 % for six consecutive runs. Accordingly, the RSDs of areas were found to be below 2.6 %. Regarding the over 20-fold reduction of the analysis time, the RSDs of the UHPLC method were still good. The peak resolution was exceptionally good for the extremely short separation. By enhancing the column temperature to 80 °C, even phenytoin and carbamazepine-10,11-epoxide were clearly baseline-separated.

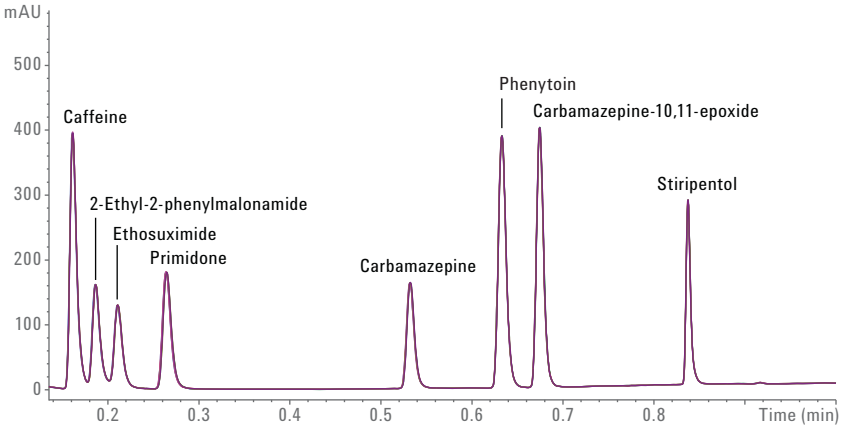
Figure 6 shows an overlay of six consecutive runs on the 2.1-mm column together with the values for RT and area precision, as well as resolution on the 1290 Infinity II LC. The RSDs of RTs were found to be below 0.1 %, except for primidone with 0.186 % for six consecutive runs. Accordingly, the RSDs of areas were found to be below 0.5 %. Regarding the over 20-fold reduction of the analysis time, the RSDs of the UHPLC method were excellent. In addition, the peak resolution was found to be equivalent. Compared to the 1290 Infinity LC, the results were improved, especially regarding precision.

In comparison to the HPLC method using a 4.6 × 150 mm, 5-µm column, an enormous time saving was possible, from 40 to 2 minutes total cycle time. With the 2.1-mm id UHPLC column, a total solvent saving of 95 % was achieved based on the short run time. In addition, only a quarter of sample amount was necessary for the analysis.



	RT RSD (%)	Area RSD (%)	Resolution
Caffeine	0.166	0.295	5.3
2-ethyl-2-phenylmalonamide	0.302	2.527	2.5
Ethosuximide	0.323	1.459	2.4
Primidone	0.318	0.229	5.0
Carbamazepine	0.057	0.265	25.7
Phenytoin	0.027	0.259	9.3
Carbamazepine-10,11-epoxide	0.017	0.206	5.0
Stiripentol	0.006	0.527	2.9

Figure 5. UHPLC analysis (optimized for speed) of seven antiepileptic drugs and caffeine (overlay of six consecutive runs) on an Agilent ZORBAX SB-C18, 2.1 × 50 mm, 1.8-µm column, using the Agilent 1290 Infinity LC system.



	RT RSD (%)	Area RSD (%)	Resolution
Caffeine	0.045	0.082	1.4
2-ethyl-2-phenylmalonamide	0.096	0.137	1.7
Ethosuximide	0.099	0.082	1.5
Primidone	0.186	0.105	3.3
Carbamazepine	0.049	0.489	14.9
Phenytoin	0.017	0.086	6.4
Carbamazepine-10,11-epoxide	0.013	0.092	2.7
Stiripentol	0.008	0.161	3.4

Figure 6. UHPLC analysis (optimized for speed) of seven antiepileptic drugs and caffeine (overlay of six consecutive runs) on an Agilent ZORBAX SB-C18, 2.1 × 50 mm, 1.8-µm column, using the Agilent 1290 Infinity II LC system.

Both systems were evaluated regarding linearity, limit of detection (LOD) and limit of quantification (LOQ). Ten different concentration levels (from 50 to 0.195 µg/mL, 1:2 dilution) were prepared from the stock solutions, and the linear relationship was determined between the peak area and the corresponding concentrations. LOD and LOQ were defined as signal-to-noise (S/N) ratios of 3:1 and 10:1 respectively.

Table 1, Table 2, and Table 3 show the results of the evaluation. All three methods showed high linearity with correlation coefficients over 0.9999 for all standards except for stiripentol on both systems.

LOD and LOQ improved over 10 times for the UHPLC method on the 2.1 × 50 mm, 1.8 µm column, and still more than five times for the ultrafast method on the 2.1-mm id column, compared to the HPLC method on the long 5-µm column. The evaluation of sensitivity showed equivalent results on both systems for the HPLC and the speed-optimized UHPLC method. For the UHPLC analysis after the simple method conversion, sensitivity was improved using the 1290 Infinity II LC.

Table 1. Comparison of linearity and sensitivity of the two LC systems for HPLC analysis.

	Linearity		LOD (ng)		LOQ (ng)	
	Agilent 1290 Infinity	Agilent 1290 Infinity II	Agilent 1290 Infinity	Agilent 1290 Infinity II	Agilent 1290 Infinty	Agilent 1290 Infinty II
Caffeine	0.99992	1	518.5	585.9	1728.3	1953.0
2-ethyl-2-phenylmalonamide	0.99995	1	1251.9	1273.7	4173.1	4245.7
Ethosuximide	0.99996	0.99993	1830.9	1723.2	6103.1	5744.1
Primidone	0.99993	1	626.0	665.8	2086.5	2219.3
Carbamazepine	0.99991	0.99999	1167.1	1220.6	3890.4	4068.8
Phenytoin	0.99990	0.99999	756.0	714.5	2520.2	2381.7
Carbamazepine-10,11-epoxide	0.99994	0.99999	751.2	837.0	2503.8	2790.0
Stiripentol	0.99961	0.99959	1024.3	1046.3	3414.3	3487.5

Table 2. Comparison of linearity and sensitivity of the two LC systems for the UHPLC method, after simple conversion.

	Linearity		LOD (ng)		LOQ (ng)	
	Agilent 1290 Infinity	Agilent 1290 Infinity II	Agilent 1290 Infinity	Agilent 1290 Infinity II	Agilent 1290 Infinity	Agilent 1290 Infinity II
Caffeine	1	0.99999	27.6	15.6	92.1	51.9
2-ethyl-2-phenylmalonamide	0.99993	0.9999	97.7	38.5	325.5	128.5
Ethosuximide	0.99997	0.99914	122.1	20.3	406.9	67.8
Primidone	1	1	33.3	19.3	111.0	64.2
Carbamazepine	1	0.99999	54.3	34.9	180.8	116.3
Phenytoin	0.99993	0.99999	30.5	14.6	101.7	48.8
Carbamazepine-10,11-epoxide	0.99995	1	32.6	15.3	108.5	50.9
Stiripentol	0.99995	0.99993	52.3	25.3	174.4	84.2

Table 3. Comparison of linearity and sensitivity of the two LC systems for ultrafast analysis, optimized for speed.

	Linearity		LOD (ng)		LOQ (ng)	
	Agilent 1290 Infinity	Agilent 1290 Infinity II	Agilent 1290 Infinity	Agilent 1290 Infinity II	Agilent 1290 Infinity	Agilent 1290 Infinity II
Caffeine	1	0.99999	31.2	45.8	103.9	152.6
2-ethyl-2-phenylmalonamide	0.99996	0.99993	114.4	109.3	381.4	364.4
Ethosuximide	0.99994	0.99917	159.7	52.3	532.4	174.4
Primidone	1	1	35.2	43.6	117.4	145.3
Carbamazepine	0.99999	0.99998	72.0	88.9	240.0	296.3
Phenytoin	0.99999	0.99999	25.0	44.9	83.3	149.8
Carbamazepine-10,11-epoxide	1	1	40.7	45.8	135.6	152.6
Stiripentol	0.99959	0.99948	49.5	47.9	164.9	159.6

Conclusion

This Application Note shows a comparison of the analysis of antiepileptic drug standards on an Agilent 1290 Infinity LC, and an Agilent 1290 Infinity II LC. The method was transferred from a standard HPLC column (4.6 × 150 mm, 5 µm) to a UHPLC column (2.1 × 50 mm, 1.8 µm). The method was transferred with a simple adjustment of flow rate, run time, and sample amount. The UHPLC was then optimized for speed, resulting in a total cycle time of 2 minutes. A significant time saving was achieved, as well as a 75 % reduction of injected sample. A total solvent saving of 95 % was achieved based on short run time. All analyses were compared regarding precision, resolution, linearity, and sensitivity for both systems. The evaluation of both HPLC and UHPLC methods revealed excellent precision, resolution, and linearity, as well as comparable LODs and LOQs. Better precision of retention time and area was found for all applied methods with the 1290 Infinity II LC system.

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