

# Analysis of Anti-epileptic Drugs with the Agilent 1290 Infinity II LC

Suitable for Agilent  
1290 Infinity III LC

## Author

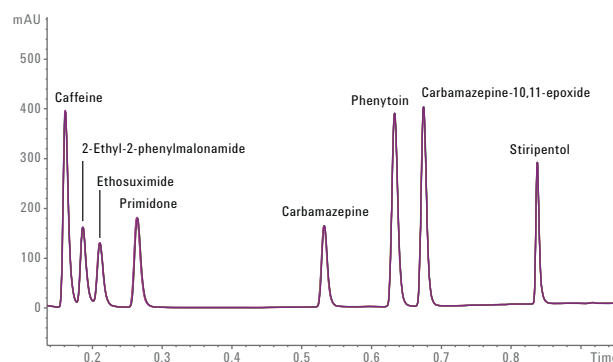
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## Application Note

Small Molecule Pharmaceuticals

## Abstract

This Application Note shows the transfer of an analysis method for anti-epileptic drugs from HPLC to UHPLC using the Agilent 1290 Infinity II LC system. An enormous time and solvent saving of approximately 95 % was achieved with an UHPLC separation optimized for speed. Using a 2.1-mm id column, the amount of injected sample could be also reduced by 75 %. Both HPLC and UHPLC methods revealed excellent precision, resolution, and linearity, as well as comparable limits of detection (LOD) and limits of quantification (LOQ).



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## Introduction

Qualitative and quantitative monitoring of pharmaceutical drugs is imperative to determine the purity and stability of ingredients under the influence of environmental factors such as temperature, humidity, and light<sup>1</sup>. This analysis is routinely executed using conventional high performance liquid chromatography (HPLC) methods, one of the most reliable and rugged analysis techniques.

Pharmaceutical laboratories have an increasing demand for highest sample throughput and lowest cost per analysis to improve their productivity of laboratory analysis. Sub-2  $\mu\text{m}$  (STM) particle technology and high-pressure systems (up to 1,300 bar) enable ultrahigh performance liquid chromatography (UHPLC) with faster analysis time. Based on the variety of new possibilities of UHPLC systems with STM columns, many classical HPLC methods are transformed to UHPLC methods.

This Application Note shows the method transfer from HPLC to UHPLC methods for the analysis of anti-epileptic drugs on the Agilent 1290 Infinity II LC system.

## Experimental

### Equipment

The deployed 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)
- 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  $\times$  150 mm, 5  $\mu\text{m}$  (p/n 883975-902)
- Agilent ZORBAX RRHT SB-C18, 4.6  $\times$  50 mm, 1.8  $\mu\text{m}$  (p/n 846975-902)
- Agilent ZORBAX RRHD SB-C18, 2.1  $\times$  50 mm, 1.8  $\mu\text{m}$  (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 typical anti-epileptic drugs (2-ethyl-2-phenylmalonamide, ethosuximide, primidone, carbamazepine, phenytoin, carbamazepine-10,11-epoxide, stiripentol, 25 ng/ $\mu\text{L}$  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- $\mu\text{m}$  membrane point-of-use cartridge (Millipak). The anti-epileptic drug standards were purchased from Sigma-Aldrich, St. Louis, Missouri, US.

## Chromatographic conditions

Table 1. Chromatographic conditions for HPLC with a 4.6  $\times$  150 mm, 5- $\mu\text{m}$  column.

Parameter	Value
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 $\mu\text{L}$
Column temperature	60 $^{\circ}\text{C}$
Detection	Signal A) 204/4 nm, reference 360/80 nm Peak width > 0.025 minutes (0.5 seconds response time) Data rate 10 Hz

Table 2. Chromatographic conditions for UHPLC with a 4.6 × 50 mm, 1.8-µm column.

Parameter	Value
Mobile phase	A) Water B) Acetonitrile
Flow rate	0.8 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	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

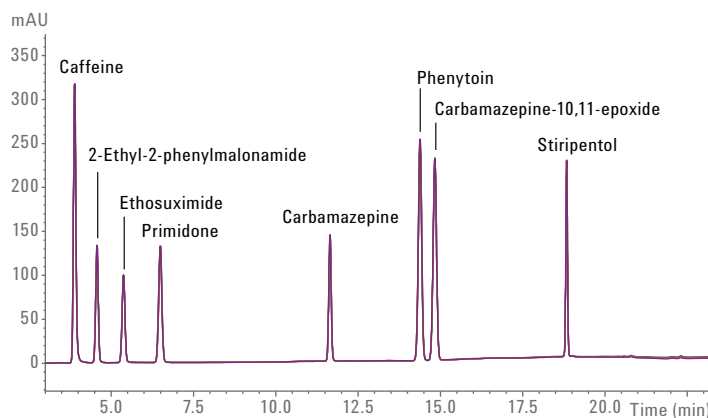
Table 3. Chromatographic conditions for UHPLC with 2.1 × 50 mm, 1.8- µm column, optimized for speed.

Parameter	Value
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 minutes
Post time	1 minutes
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

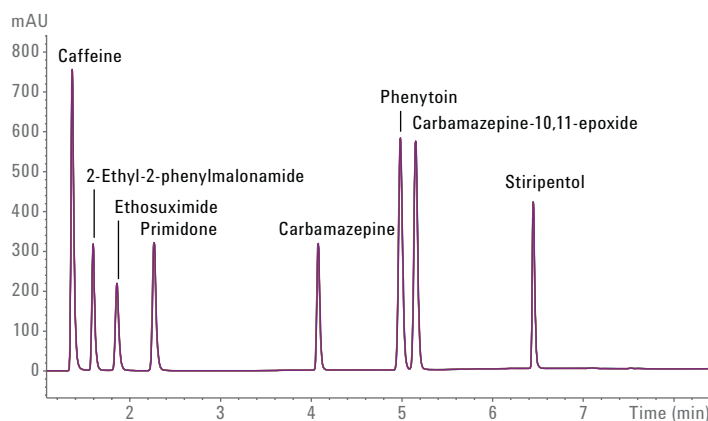
The anti-epileptic drug standards were analyzed under HPLC conditions (Figure 1). Six consecutive runs were analyzed for their precision regarding retention time (RT) and area, as well as for resolution. The relative standard deviations (RSD) of retention time and area were found to be excellent, below 0.042 and 0.15 % respectively.

To shorten the analysis time of the drug standards, the method was transferred to UHPLC using an Agilent ZORBAX SB-C18, 4.6 × 50 mm, 1.8-μm column. The total cycle time was shortened from 40 to 13.5 minutes, resulting in a total time and solvent saving of over 66 %. Figure 2 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. The RSDs for RT and area were found to be excellent, with values below 0.085 and 0.42 % respectively. Also, the resolution was still comparable to the HPLC method.



	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 1. HPLC Analysis of seven anti-epileptic drugs plus caffeine (overlay of six consecutive runs) on an Agilent ZORBAX SB-C18, 4.6 × 150 mm, 5-μm column.



	RT RSD (%)	Area RSD (%)	Resolution
Caffeine	0.067	0.284	1.9
2-Ethyl-2-phenylmalonamide	0.074	0.249	4.1
Ethosuximide	0.067	0.266	4.1
Primidone	0.084	0.289	5.8
Carbamazepine	0.033	0.413	23.1
Phenytoin	0.026	0.248	12.7
Carbamazepine-10,11-epoxide	0.023	0.314	2.1
Stiripentol	0.008	0.136	5.2

Figure 2. UHPLC Analysis of seven anti-epileptic drugs plus caffeine (overlay of six consecutive runs) on an Agilent ZORBAX SB-C18, 4.6 × 50 mm, 1.8-μm column.

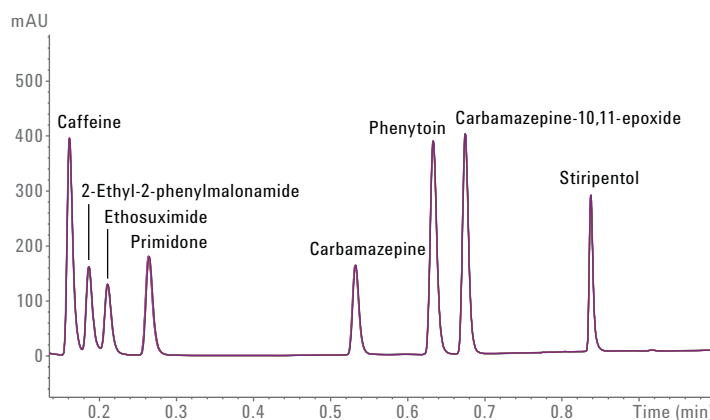
To achieve an even shorter, ultrafast separation to save more time, solvents, and sample, as well as reducing the sample volume injected, an Agilent ZORBAX SB-C18, 2.1 × 50 mm, 1.8-μm column was used. With an UHPLC method optimized for speed, a separation within 0.9 minutes was possible with a flow rate of 1.5 mL/min. Figure 3 shows an overlay of six consecutive runs on the 2.1-mm column together with the values for RT and area precision and resolution.

The RSDs of retention times were found to be below 0.1 %, except for primidone with 0.186 %, for six consecutive runs. The RSDs of areas were found to be below 0.5 % for six consecutive runs. Regarding the more than 20-fold reduction of analysis time, the RSDs of the UHPLC method were excellent. The peak resolution was still good for the ultrafast separation time. By increasing the column temperature to 80 °C, even phenytoin and carbamazepine-10,11-epoxide were clearly baseline-separated.

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 the sample amount was necessary for the analysis.

All three methods 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 4 shows the results of the evaluation. All three methods showed high linearity, with correlation coefficients over 0.9999 for all standards except stiripentol.



	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 3. UHPLC Analysis of seven anti-epileptic drugs plus caffeine (overlay of six consecutive runs) on an Agilent ZORBAX SB-C18, 2.1 × 50 mm, 1.8-μm column.

Table 4. Comparison of linearity of the HPLC and the two UHPLC methods.

	Correlation coefficient R 4.6 × 150 mm, 5 μm	Correlation coefficient R 4.6 × 50 mm, 1.8 μm	Correlation coefficient R 2.1 × 50 mm, 1.8 μm
Caffeine	1	0.99999	0.99999
2-Ethyl-2-phenylmalonamide	1	0.99999	0.99993
Ethosuximide	0.99993	0.99995	0.99994
Primidone	1	0.99999	1
Carbamazepine	0.99999	1	0.99998
Phenytoin	0.99999	0.99997	0.99999
Carbamazepine-10,11-epoxide	0.99999	0.99999	1
Stiripentol	0.99959	0.99997	0.99948

LOD and LOQ improved over 100 times for the UHPLC method on the 4.6 × 50 mm, 1.8-μm column, and more than 10 times for the ultrafast method on the 2.1-mm id column compared to the HPLC column on the long 5-μm column (Tables 5 and 6.)

## Conclusion

This Application Note shows the analysis of anti-epileptic drug standards on an Agilent 1290 Infinity II LC system. The method was transferred from a standard HPLC column (4.6 × 150 mm, 5 μm) to two short UHPLC columns (4.6 and 2.1 × 50 mm, 1.8 μm). The first transfer to the 4.6 × 50 mm, 1.8-μm column achieved a time and solvent saving of over 65 %. The separation on the 2.1 × 50 mm, 1.8-μm column was optimized for speed with a flow rate of 1.5 mL/min, resulting in a total cycle time of 2 minutes. Hence, an enormous time saving was achieved as well as a 75 % reduction of injected sample. Ultimately, a total solvent saving of 95 % was achieved based on the short run time. The evaluation of both HPLC and UHPLC methods revealed excellent precision, resolution, and linearity, as well as comparable LODs and LOQs.

Table 5. Comparison of LODs in nanograms as amount on-column of the HPLC and the two UHPLC methods.

	LOD (ng) 4.6 × 150 mm, 5 μm	LOD (ng) 4.6 × 50 mm, 1.8 μm	LOD (ng) 2.1 × 50 mm, 1.8 μm
Caffeine	585.9	3.9	45.8
2-Ethyl-2-phenylmalonamide	1273.7	9.5	109.3
Ethosuximide	1723.2	9.3	52.3
Primidone	665.8	6.8	43.6
Carbamazepine	1220.6	14.2	88.9
Phenytoin	714.5	7.7	44.9
Carbamazepine-10,11-epoxide	837.0	8.2	45.8
Stiripentol	1046.3	11.2	47.9

Table 6. Comparison of LOQs in nanograms as amount on-column of the HPLC and the two UHPLC methods.

	LOQ (ng) 4.6 × 150 mm, 5 μm	LOQ (ng) 4.6 × 50 mm, 1.8 μm	LOQ (ng) 2.1 × 50 mm, 1.8 μm
Caffeine	1953.0	12.9	152.6
2-Ethyl-2-phenylmalonamide	4245.7	31.6	364.4
Ethosuximide	5744.1	30.9	174.4
Primidone	2219.3	22.8	145.3
Carbamazepine	4068.8	47.2	296.3
Phenytoin	2381.7	25.6	149.8
Carbamazepine-10,11-epoxide	2790.0	27.2	152.6
Stiripentol	3487.5	37.4	159.6

## Reference

1. Darwish, H. W; *et al.* A Stability-Indicating HPLC-DAD Method for Determination of Stiripentol: Development, Validation, Kinetics, Structure Elucidation and Application to Commercial Dosage Form. *Journal of Analytical Methods in Chemistry* **2004**, Volume 2014, Article ID 638951.

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