Application Note

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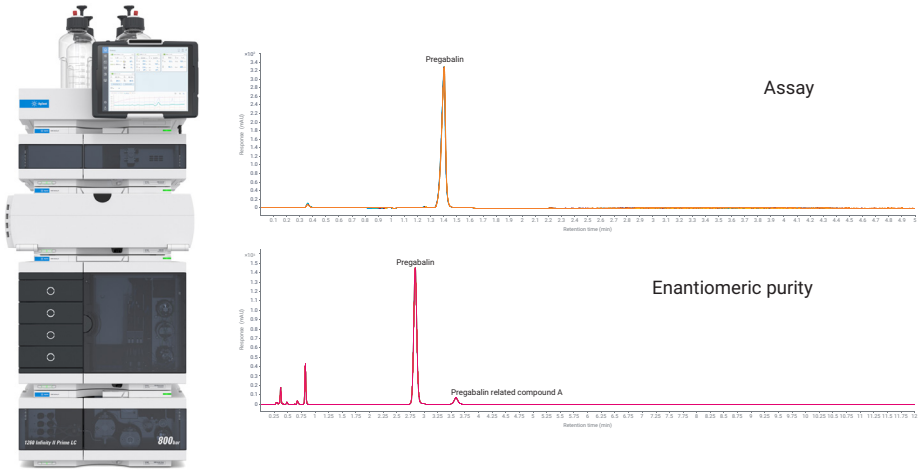
Two USP Analyses of Pregabalin Using One LC

Assay and enantiomeric purity analysis using the Agilent 1260 Infinity II Prime LC with automated column selection



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Abstract

In the United States Pharmacopeia (USP), the monograph on pregabalin states different high performance liquid chromatography (HPLC) methods for the assay and the determination of enantiomeric purity. This application note describes the performance of the assay as well as the determination of enantiomeric purity according to USP on the Agilent 1260 Infinity II Prime LC with a column selection valve. Both methods can be performed on one LC system with no manual intervention required. Furthermore, the assay and determination of enantiomeric purity for pregabalin are transferred to UHPLC conditions in accordance with USP guidelines, chapter 621, enabling time and solvent savings.

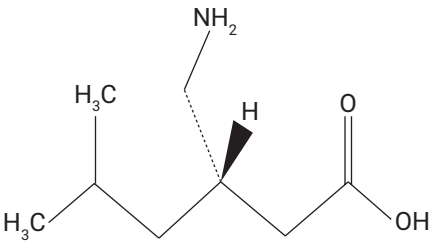
Introduction

Pregabalin (see Figure 1), marketed under the trade name Lyrica and other names, is among the top 15 pharmaceutical products in worldwide sales. This product had a sales volume of around 5 billion dollars in 2018.[1] Pregabalin is used in cases of neuropathic pain and as an adjunctive pharmaceutical for epilepsy.[2,3]

The monograph on pregabalin from the United States Pharmacopeia (USP) states different high performance liquid chromatography (HPLC) methods for the assay, the determination of organic impurities, and the determination of enantiomeric purity.[4]

This application note demonstrates the assay and determination of enantiomeric purity for pregabalin on one LC system, employing the Agilent 1260 Infinity II Prime LC equipped with a column selection valve. The combination of the Agilent 1260 Infinity II Flexible Pump with a column selection valve enables the automation of running two USP methods on one LC. This combination of methods saves time and avoids errors, as no manual interaction is required between running the two methods.

Furthermore, the pressure range of up to 800 bar offered by the 1260 Infinity II Prime LC allows the transfer of the pregabalin assay and determination of enantiomeric purity for pregabalin to UHPLC conditions in accordance with USP guidelines, chapter 621. This results in time and solvent savings and enables further cost reduction.



Pregabalin,
(S)-3-(Aminomethyl)-5-methylhexanoic acid

Figure 1. Structure of pregabalin.

Experimental

Equipment

The Agilent 1260 Infinity II Prime LC System comprised the following modules:

- Agilent 1260 Infinity II Flexible Pump (G7104C)
- Agilent 1260 Infinity II Multisampler (G7167A) with sample thermostat (option #101)
- Agilent 1260 Infinity II Multicolumn Thermostat (G7116A) with valve drive installed (option #058) equipped with 4-position/10-port column selection valve (G4237A)
- Agilent 1260 Infinity II Diode Array Detector HS (G7117C) with Max-Light cartridge cell, 10 mm (G4212-60008)

Software

Agilent OpenLab CDS Version 2.4.

Columns

Agilent ZORBAX Eclipse Plus C18, 4.6 × 250 mm, 5 µm (p/n 959990-902)

Agilent ZORBAX Eclipse Plus C18, 2.1 × 100 mm, 1.8 µm (p/n 959758-902)

Agilent InfinityLab Poroshell 120 EC-C18, 2.1 × 100 mm, 2.7 µm, 1,000 bar (p/n 695575-902)

Chemicals

All solvents were LC grade. Acetonitrile and acetone were purchased from Merck (Darmstadt, Germany). Fresh ultrapure water was obtained from a Milli-Q Integral system equipped with a 0.22 µm membrane point-of-use cartridge (Millipak, EMD Millipore, Billerica, MA, USA). Pregabalin and pregabalin related compound A (European Pharmacopoeia reference standards) were purchased from EDQM (Strasbourg, France). Marfey's reagent (Na-(2,4-dinitro-5-fluorophenyl)-L-alanine amide), sodium bicarbonate, and triethylamine were obtained from Sigma-Aldrich (Steinheim, Germany). Phosphoric acid was purchased from Merck (Darmstadt, Germany).

Pregabalin assay according to USP

Standard solution:

A 2.0 mg/mL pregabalin standard solution was obtained by dissolution of 20 mg pregabalin to 10 mL with water/acetonitrile (95/5, v/v).

Method:

Table 1. Pregabalin assay.

Parameter	Value
Column	ZORBAX Eclipse Plus C18, 4.6 × 250 mm, 5 µm
Solvent	A: Water/acetonitrile (95/5, v/v)
Gradient	100% A, isocratic Stop time: 25 min Post time: Off
Flow Rate	1.000 mL/min
Temperature	25 °C
Detection	205 nm/4 nm, reference 360 nm/100 nm 10 Hz
Injection	Injection volume: 20 µL Sample temperature: 10 °C Needle wash: 3 s in water/acetonitrile (90/10)

Table 2. Pregabalin assay – transfer to UHPLC.

Parameter	Value
Column	ZORBAX Eclipse Plus C18, 2.1 × 100 mm, 1.8 µm
Solvent	A: Water/acetonitrile (95/5, v/v)
Gradient	100% A, isocratic Stop time: 5 min Post time: Off
Flow Rate	0.579 mL/min
Temperature	25 °C
Detection	205 nm/4 nm, reference 360 nm/100 nm 40 Hz
Injection	Injection volume: 5 µL Sample temperature: 10 °C Needle wash: 3 s in water/acetonitrile (90/10)

Pregabalin enantiomeric purity according to USP

Sensitivity stock solution: A 0.5 µg/mL pregabalin sensitivity stock solution was prepared as follows: 5 mg pregabalin were dissolved in 50 mL water and diluted to 100 mL volume with acetone (resulting concentration 50 µg/mL). 1 mL of this solution was diluted with 50 mL water and further diluted to 100 mL volume with acetone (resulting concentration 0.5 µg/mL).

Standard stock solution: A standard stock solution containing 1.0 mg/mL pregabalin and 0.05 mg/mL pregabalin related compound A was prepared by dissolution of 20 mg pregabalin and 1 mg pregabalin related compound A in 10 mL water and dilution to 20 mL volume with acetone.

Sensitivity solution: A 1 mL aliquot of the sensitivity stock solution was transferred to a 10 mL volumetric flask. A 0.6 mL aliquot of derivatizing reagent solution (3 mg/mL Marfey's reagent in acetone) and 100 µL 1 M sodium bicarbonate were added to the solution. The sensitivity solution was heated at 55 °C in a water bath for one hour. The solution was allowed to cool to room temperature and diluted to volume with water.

Standard solution: A 1 mL aliquot of the standard stock solution was transferred to a 10 mL volumetric flask. A 0.6 mL aliquot of derivatizing reagent solution (3 mg/mL Marfey's reagent in acetone) and 100 µL 1 M sodium bicarbonate were added to the solution. The standard solution was heated at 55 °C in a water bath for one hour. The solution was allowed to cool to room temperature and diluted to volume with water.

Method:

Table 3. Pregabalin enantiomeric purity.

Parameter	Value
Column	ZORBAX Eclipse Plus C18, 4.6 × 250 mm, 5 µm
Solvent	B: Triethylamine and water (7.2/1000, v/v), adjusted to pH 3.0 with 50% phosphoric acid/acetonitrile (62/38, v/v)
Gradient	100% B, isocratic Stop time: 52 min Post time: Off
Flow Rate	2.000 mL/min
Temperature	25 °C
Detection	340 nm/4 nm, no reference 10 Hz
Injection	Injection volume: 20 µL Sample temperature: 10 °C Needle wash: 3 s in water/acetonitrile (90/10)

Table 4. Pregabalin enantiomeric purity – transfer to UHPLC employing a sub-2-µm column.

Parameter	Value
Column	ZORBAX Eclipse Plus C18, 2.1 × 100 mm, 1.8 µm
Solvent	B: Triethylamine and water (7.2/1000, v/v), adjusted to pH 3.0 with 50% phosphoric acid/acetonitrile (62/38, v/v)
Gradient	100% B, isocratic Stop time: 15 min Post time: Off
Flow Rate	0.650 mL/min
Temperature	25 °C
Detection	340 nm/4 nm, no reference 40 Hz
Injection	Injection volume: 5 µL Sample temperature: 10 °C Needle wash: 3 s in water/acetonitrile (90/10)

Table 5. Pregabalin enantiomeric purity – transfer to UHPLC employing a superficially porous column.

Parameter	Value
Column	InfinityLab Poroshell 120 EC-C18 2.1 × 100 mm, 2.7 µm, 1,000 bar
Solvent	B: Triethylamine and water (7.2/1000, v/v), adjusted to pH 3.0 with 50% phosphoric acid/acetonitrile (62/38, v/v)
Gradient	100% B, isocratic Stop time: 12 min Post time: Off
Flow Rate	0.772 mL/min
Temperature	25 °C
Detection	340 nm/4 nm, no reference 40 Hz
Injection	Injection volume: 5 µL Sample temperature: 10 °C Needle wash: 3 s in water/acetonitrile (90/10)

Results and discussion

Employing the 1260 Infinity II Prime LC equipped with a column selection valve, the assay and determination of enantiomeric purity for pregabalin according to USP are performed on one LC system. The single LC system with automated column switching eliminates manual intervention between running the two methods.

Figure 2 shows a schematic representation of the configuration of the 4-position/10-port column selection valve. The column employed in the pregabalin assay is installed in position one and the column employed for determination of enantiomeric purity for pregabalin is installed in position two. The required column is then selected in the acquisition method. A bypass and waste position can be employed for solvent switching and flushing in between methods.

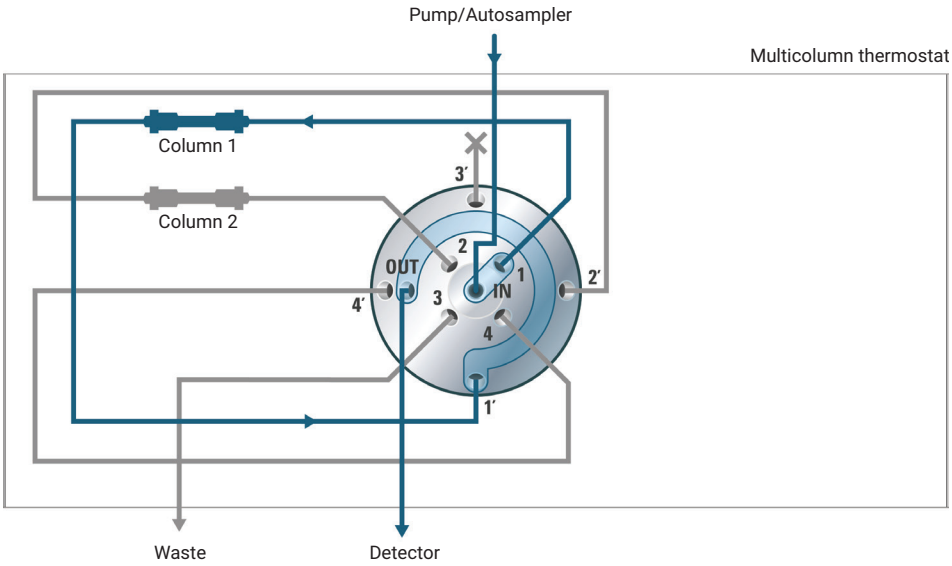


Figure 2. Schematic representation of the 4-position/10-port column selection valve with two columns installed.

Pregabalin assay according to USP

The pregabalin assay according to USP is performed using an Eclipse Plus C18, 4.6 × 250 mm, 5 µm column at a flow rate of 1.0 mL/min. Figure 3 shows the analysis of the pregabalin standard solution. Excellent retention time and peak area precision are obtained, easily fulfilling the USP system suitability requirement of relative standard deviation not more than (NMT) 0.73%. The pregabalin peak shows a tailing factor of 0.8, fulfilling the USP system suitability requirement of a tailing factor NMT 1.5.

The pressure range of up to 800 bar offered by the 1260 Infinity II Prime LC, allows the transfer of the pregabalin assay to UHPLC conditions. In accordance with USP guidelines, chapter 621, the method can be transferred to an Eclipse Plus C18, 2.1 × 100 mm, 1.8 µm column. This transfer can occur since the length to particle size ratio is within –25 to 50% of the prescribed column. The flow rate is changed to 0.579 mL/min due to the change in column diameter and particle size. The injection volume is adjusted to the change in column diameter.

The UHPLC analysis of the pregabalin standard solution is shown in Figure 4.

Under UHPLC conditions, excellent retention time and peak area precision are observed. The tailing factor of 0.8 of the pregabalin peak is maintained.

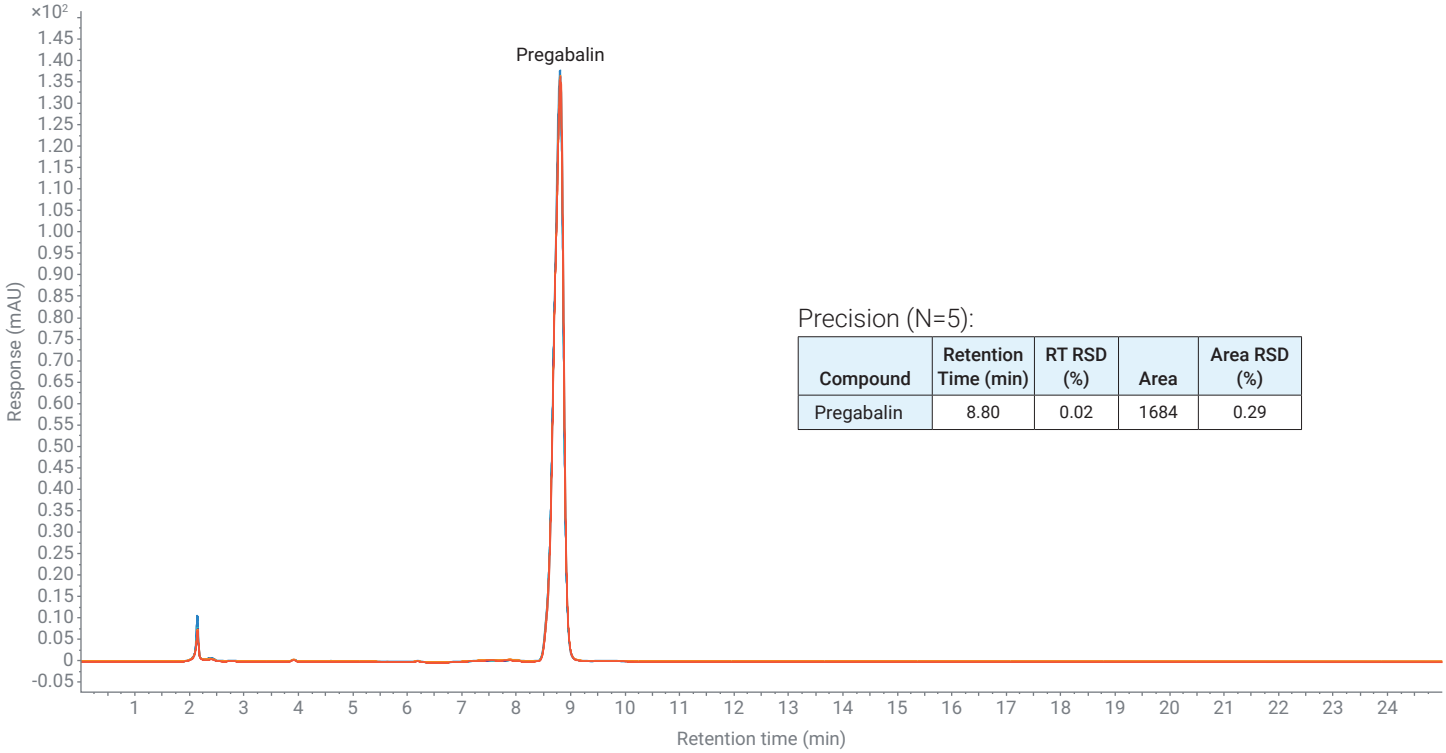
The transfer to UHPLC conditions enables the pregabalin assay to deliver an 80% time saving and 88% solvent saving.

Pregabalin enantiomeric purity according to USP

The determination of enantiomeric purity for pregabalin according to USP is shown in Figure 5. The analysis was performed using an Eclipse Plus C18, 4.6 × 250 mm, 5 µm column at a flow rate of 2.0 mL/min. The peaks eluting before pregabalin clearly originate from the derivatization performed, as they are also observed in the analysis of a blank sample subjected to derivatization.

Figure 6 shows the results from the system suitability analyses for determination of enantiomeric purity for pregabalin according to USP. In the analysis of the sensitivity solution, a signal-to-noise (S/N) ratio of 19.4 is observed. This S/N ratio fulfills the USP system suitability requirement of a S/N ratio not less than (NLT) 10 (Figure 6A). In the analysis of the standard solution, pregabalin and pregabalin related compound A are separated with a resolution of 6.5 (Figure 6B). According to the USP system suitability requirements, a resolution of NLT 3.0 between pregabalin and pregabalin related compound A is required. Excellent retention time and peak area precision are observed for pregabalin and pregabalin related compound A in the analysis of the standard solution (Figure 6B). Furthermore, the USP system suitability requirement of relative standard deviation NMT 5.0% for pregabalin related compound A is fulfilled.

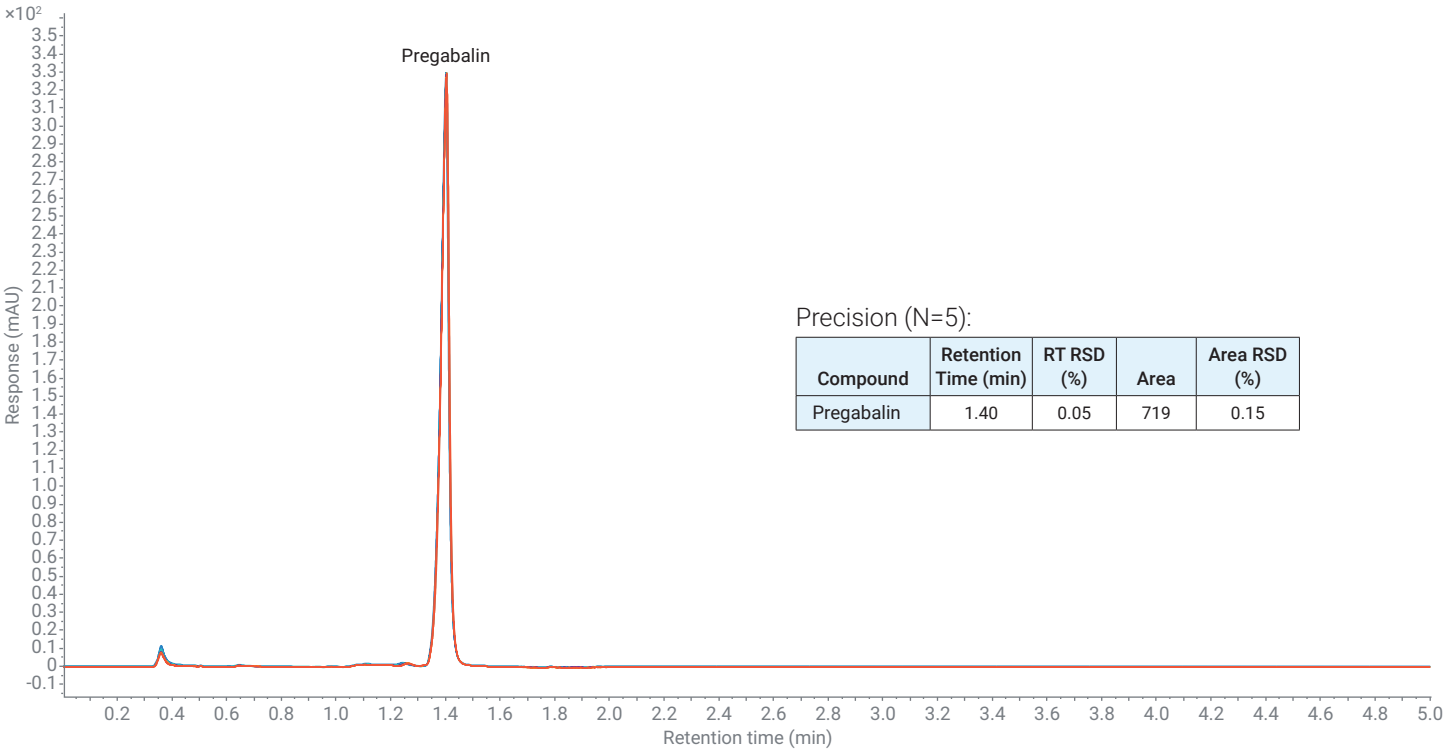
To enable time and solvent saving, the determination of enantiomeric purity for pregabalin according to USP is also transferred to UHPLC conditions in accordance with USP guidelines, chapter 621. The method can be transferred to an Eclipse Plus C18, 2.1 × 100 mm, 1.8 µm column. This column can be operated at a flow rate of 0.650 mL/min within the pressure range of the 1260 Infinity II Prime LC. Excellent retention time and peak area precision are obtained and the resolution between pregabalin and pregabalin related compound A increased to 7.8 (see Figure 7). The determination of enantiomeric purity for pregabalin under UHPLC conditions employing an Eclipse Plus C18, 2.1 × 100 mm, 1.8 µm column fulfills all USP system suitability requirements. The UHPLC method also enables 71% time saving and 90% solvent saving compared to the HPLC method.



Precision (N=5):

Compound	Retention Time (min)	RT RSD (%)	Area	Area RSD (%)
Pregabalin	8.80	0.02	1684	0.29

Figure 3. Pregabalin assay according to USP: Analysis of the pregabalin standard solution (N=5).



Precision (N=5):

Compound	Retention Time (min)	RT RSD (%)	Area	Area RSD (%)
Pregabalin	1.40	0.05	719	0.15

Figure 4. Pregabalin assay according to USP: UHPLC analysis of the pregabalin standard solution (N=5).

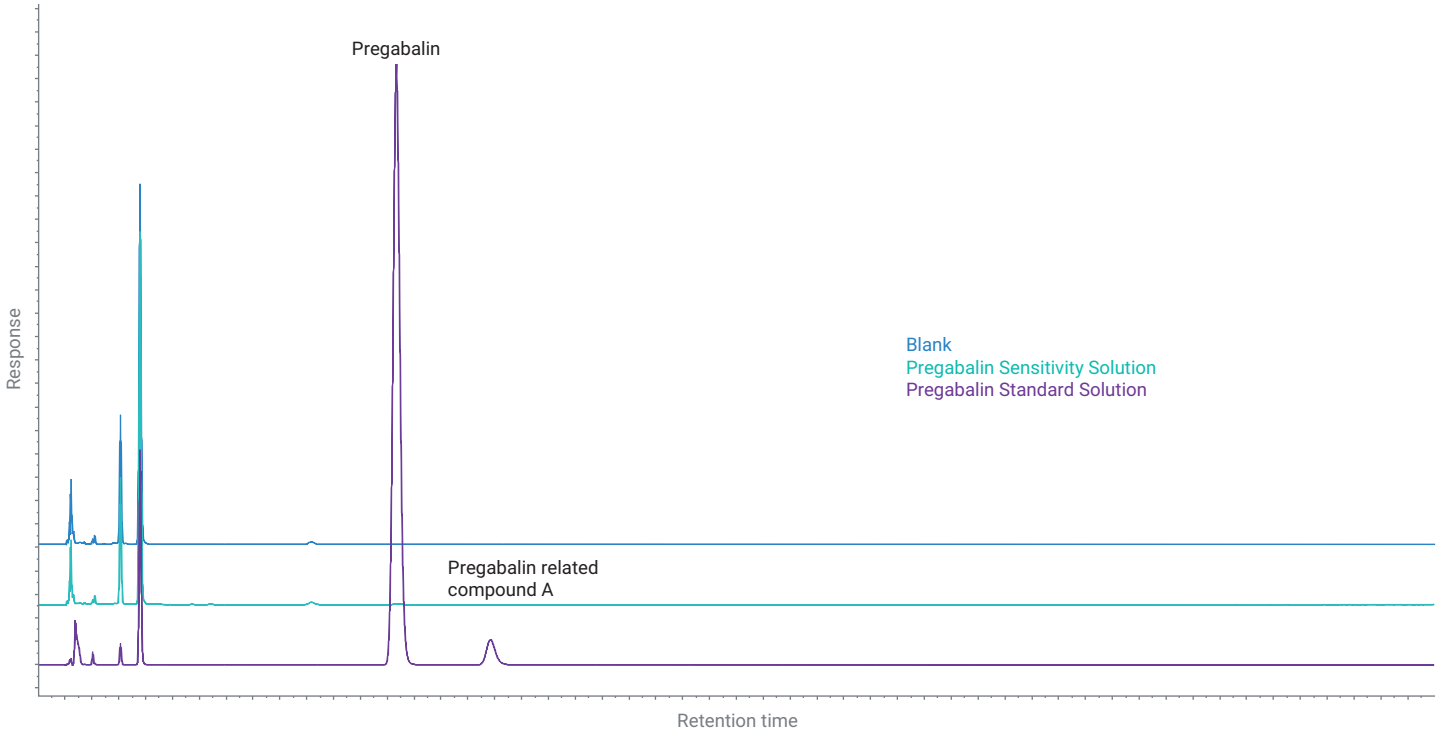
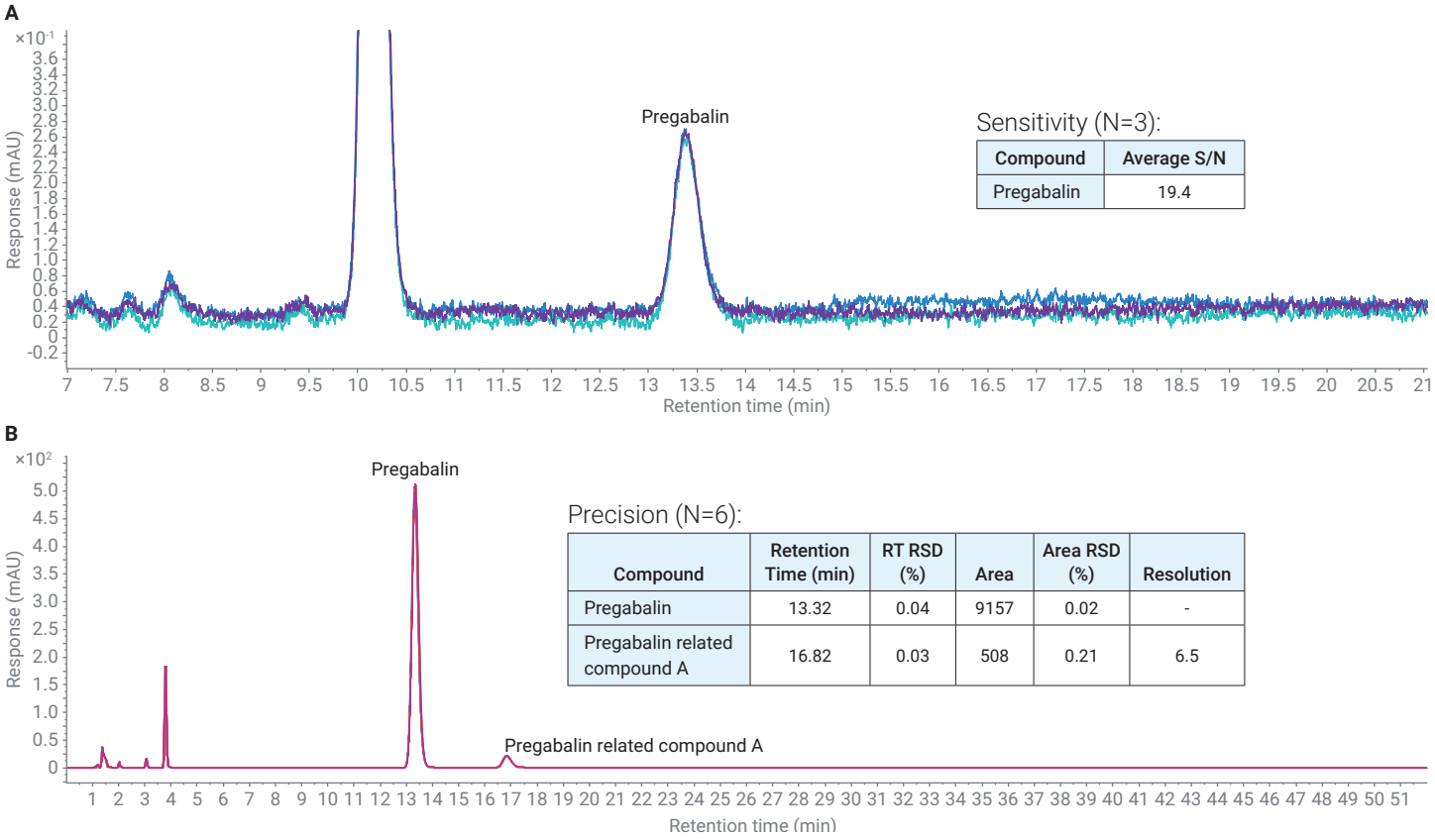


Figure 5. Determination of enantiomeric purity for pregabalin according to USP: Overlay a blank run (blue) and analysis of the pregabalin sensitivity solution (turquoise) and the pregabalin standard solution (purple).



Compound	Average S/N
Pregabalin	19.4

Compound	Retention Time (min)	RT RSD (%)	Area	Area RSD (%)	Resolution
Pregabalin	13.32	0.04	9157	0.02	-
Pregabalin related compound A	16.82	0.03	508	0.21	6.5

Figure 6. Determination of enantiomeric purity for pregabalin according to USP; (A) analysis of the sensitivity solution (N=3); (B) analysis of the standard solution (N=6).

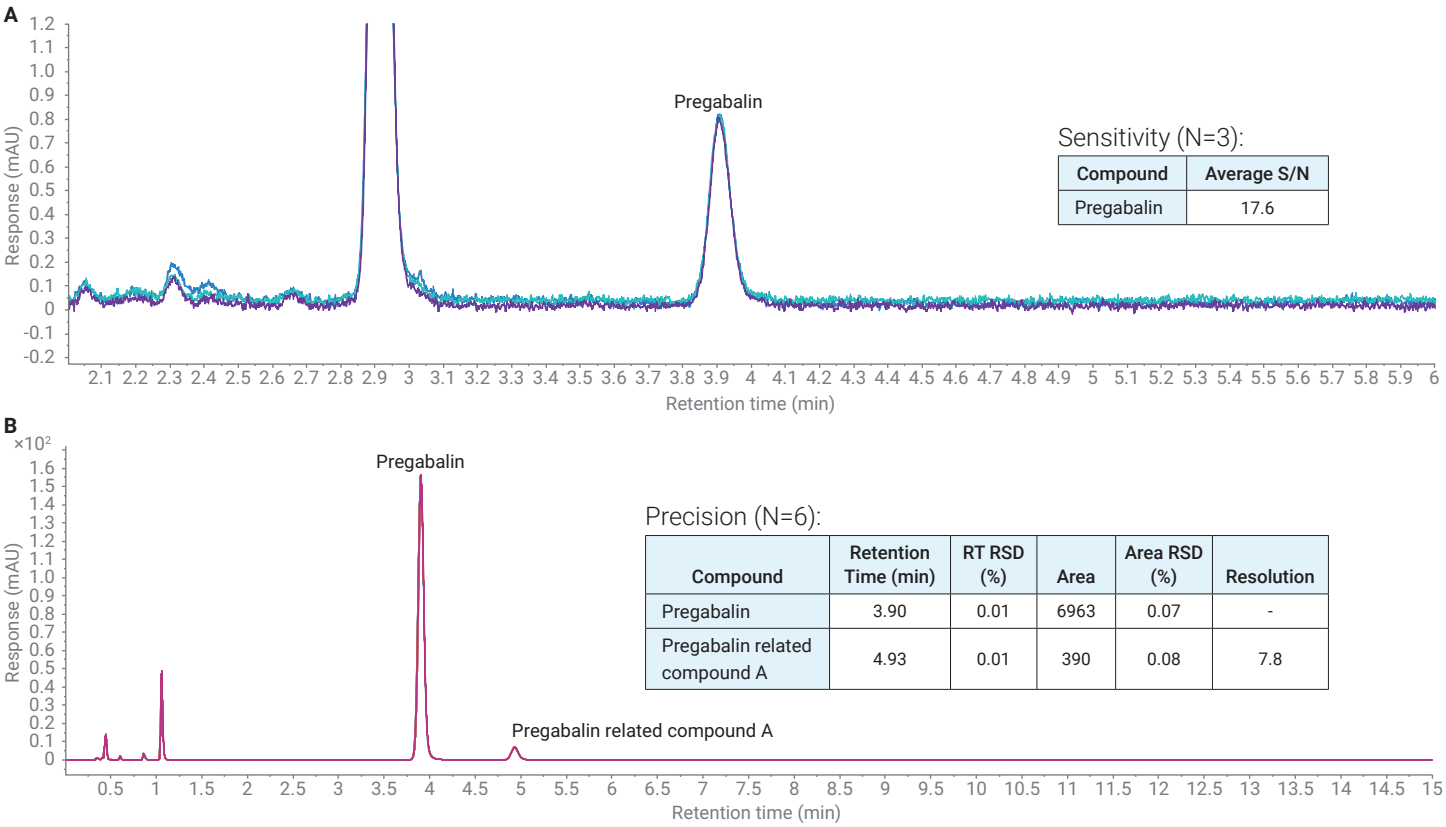


Figure 7. UHPLC determination of enantiomeric purity for pregabalin according to USP employing an Eclipse Plus C18, 2.1 x 100 mm, 1.8 µm column; (A) analysis of the sensitivity solution (N=3); (B) analysis of the standard solution (N=6).

There is a second option for transferring the determination of enantiomeric purity for pregabalin to UHPLC conditions in accordance with USP guidelines, chapter 621. It is the transfer to an InfinityLab Poroshell 120 EC-C18, 2.1 × 100 mm, 2.7 µm, 1,000 bar column, operated at a flow rate of 0.772 mL/min. This transfer is possible, because the number of theoretical plates (N) is within −25 to 50% relative to the prescribed column. Figure 8 shows the UHPLC determination of enantiomeric purity for pregabalin according to USP employing an InfinityLab Poroshell 120 EC-C18, 2.1 × 100 mm, 2.7 µm, 1,000 bar column. Excellent retention time and peak area precision are obtained and all USP system suitability requirements are fulfilled. Compared to the HPLC method, the UHPLC determination of enantiomeric purity for pregabalin employing an InfinityLab Poroshell 120 EC-C18, 2.1 × 100 mm, 2.7 µm, 1,000 bar column enables 77% time saving and 91% solvent saving.

Conclusion

The Agilent 1260 Infinity II Prime LC equipped with a column selection valve enables the automation of the assay and determination of enantiomeric purity for pregabalin, according to USP on one LC system with no interaction required. Excellent retention time and peak area precision are obtained for both methods and all USP system suitability requirements are fulfilled. Furthermore, the assay and determination of enantiomeric purity for pregabalin can be transferred to UHPLC conditions in accordance with USP guidelines, chapter 621. The method transfer is facilitated by the pressure range of up to 800 bar offered by the 1260 Infinity II Prime LC. The transfer of both methods to UHPLC conditions reduces the run time for both methods from 77 to 17 minutes and delivers a 90% solvent saving.

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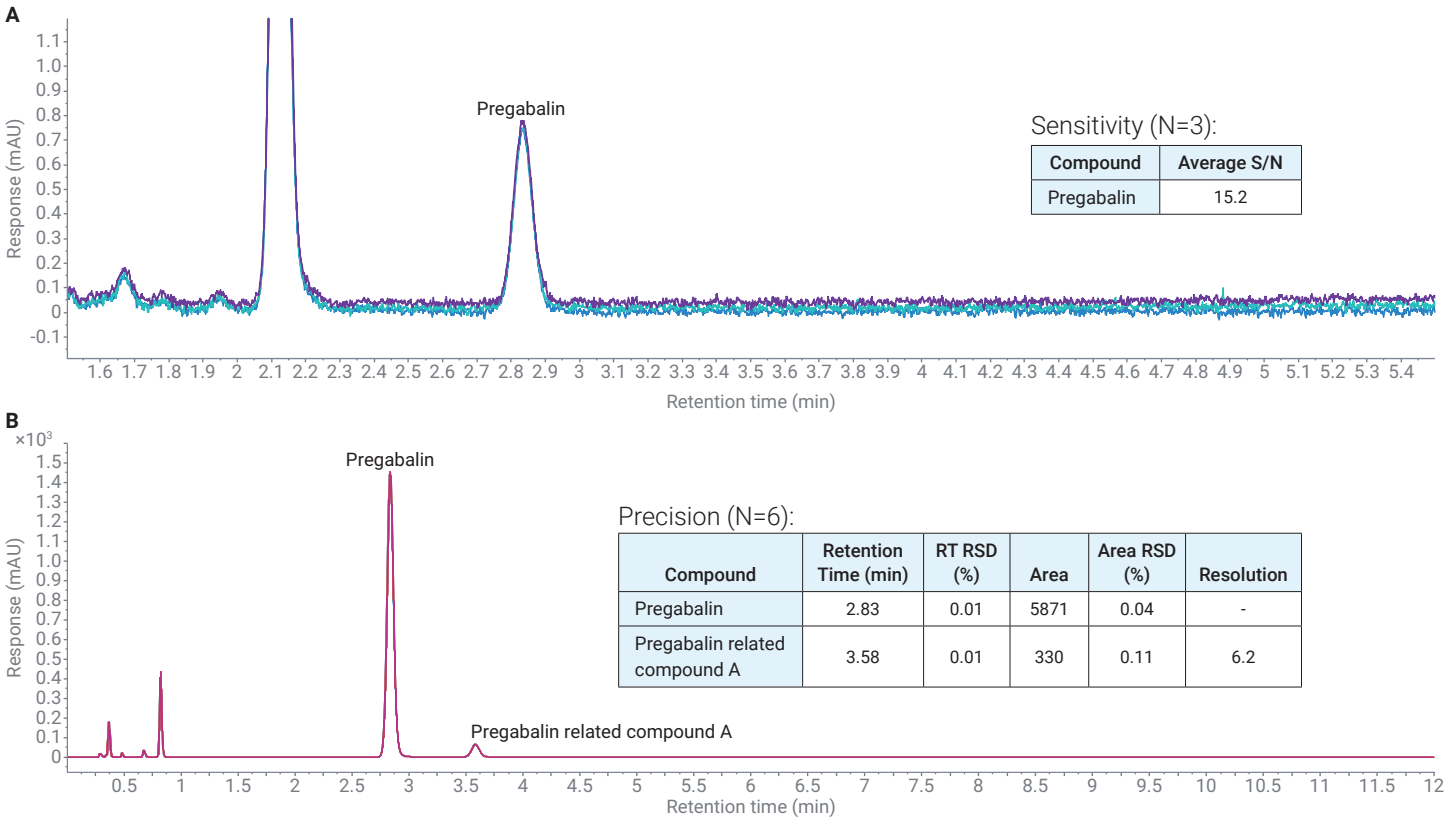

Figure 8. UHPLC determination of enantiomeric purity for pregabalin according to USP employing an InfinityLab Poroshell 120 EC-C18, 2.1 x 100 mm, 2.7 µm, 1,000 bar column; (A) analysis of the sensitivity solution (N=3); (B) analysis of the standard solution (N=6).

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