APPLICATION BRIEF

Liquid Chromatography

AUTHORS

Sagar Atugade
PerkinElmer, Inc.
Mumbai, India

Suma Ramagiri, Ph.D.
PerkinElmer, Inc.
Waltham, MA USA

HPLC Analysis of Pregabalin Using Epic C18 Column in Accordance With the United States Pharmacopeia

Introduction

Pregabalin, shown in Figure 1, is an anti-epileptic drug, also called an anticonvulsant. It works by slowing down impulses in the brain that cause seizures. Pregabalin also affects chemicals in the brain that send pain signals across the nervous system. Pregabalin is used to treat pain caused by fibromyalgia, or nerve pain in people with diabetes (diabetic neuropathy), herpes zoster (post-herpetic neuralgia), or spinal cord injury.[1]

The United States Pharmacopeia (USP) provides monographs for identification and impurity testing of the drug substance.[2,3] This application brief describes the analysis of Pregabalin, using a PerkinElmer Epic™ C18 column and the newly released LC 300 liquid chromatography system with SimplicityChrom™ chromatography data system (CDS) for the analysis of Pregabalin in accordance with the USP.

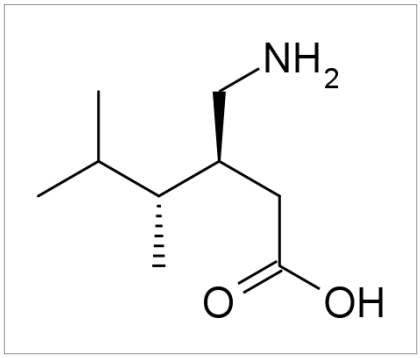

Figure 1: Chemical structure of Pregabalin.

PerkinElmer®
For the Better

Experimental

All LC method parameters are shown in Table 1.

Table 1: LC Method Parameters.

Instrument	PerkinElmer LC 300 UHPLC system with Photodiode Array (PDA) detector
Column	Epic C18 250 x 4.6 mm, 5 µm (P/N: 155291-EC18)
Mobile Phase	Acetonitrile and water (5:95)
Flow Rate	1 mL/min
Column Temp	25°C
Wavelength	Acquisition 205 nm, bandwidth 10 nm, reference 400 nm, bandwidth 10 nm
Injection Volume	20 µL (full loop)
Autosampler Temp	10°C

Solvents, Standards, and Samples

All solvents used in this work were HPLC grade, and samples were filtered using a 0.45 µm nylon filter (P/N: 02542903). A standard solution of USP Pregabalin (2.0 mg/mL) was prepared using mobile phase as the diluent.

Results and Discussion

The USP monograph specifies the use of an L1 column (250 x 4.6 mm, 5 µm). This is defined as octadecyl silane chemically bonded to porous silica or ceramic micro-particles, 3 to 10 µm in diameter. The Epic C18 column complies with the USP monograph and is ideal for the separation of Pregabalin as can be seen in the results, Table 2.

Table 2. Results Summary. RSD calculated from five successive injections.

Suitability Parameter	Mobile Phase A (%)	Mobile Phase B (%)
Peak Area RSD (%)	0.60	0.73
Retention Time RSD (%)	0.16	0.73
Tailing Factor (5% Peak Height)	1.0	1.5

The analysis of Pregabalin was carried out using an Epic C18 (250 x 4.6 mm, 5 µm) column (P/N: 155291-EC18) in under 30 minutes (RT-10.43 minutes), as detailed in Figure 2. The USP monograph requires that the relative standard deviation (RSD) for peak area and RT of five replicate injections be no more than 0.73%, and that the tailing factor be no more than 1.5. The Epic C18 column met both requirements (Table 2). These results demonstrate that the Epic C18 column, along with the LC 300 UHPLC system, are appropriate for this method.

Conclusion

The Epic C18 column is well suited for the identification of Pregabalin following the USP monograph method, as the tailing factor and area RSD achieved are well within the acceptance criteria. The LC 300 and Epic C18 column provide excellent repeatability between injections, as shown in Figure 3. The superior base deactivation of the Epic column stationary phase provided excellent peak shapes.

References

1. https://www.drugs.com/mtm/pregabalin.html.
2. USP-NF <621> Chromatography general chapter.
3. Pregabalin, USP-43-NF38, United States Pharmacopeia.

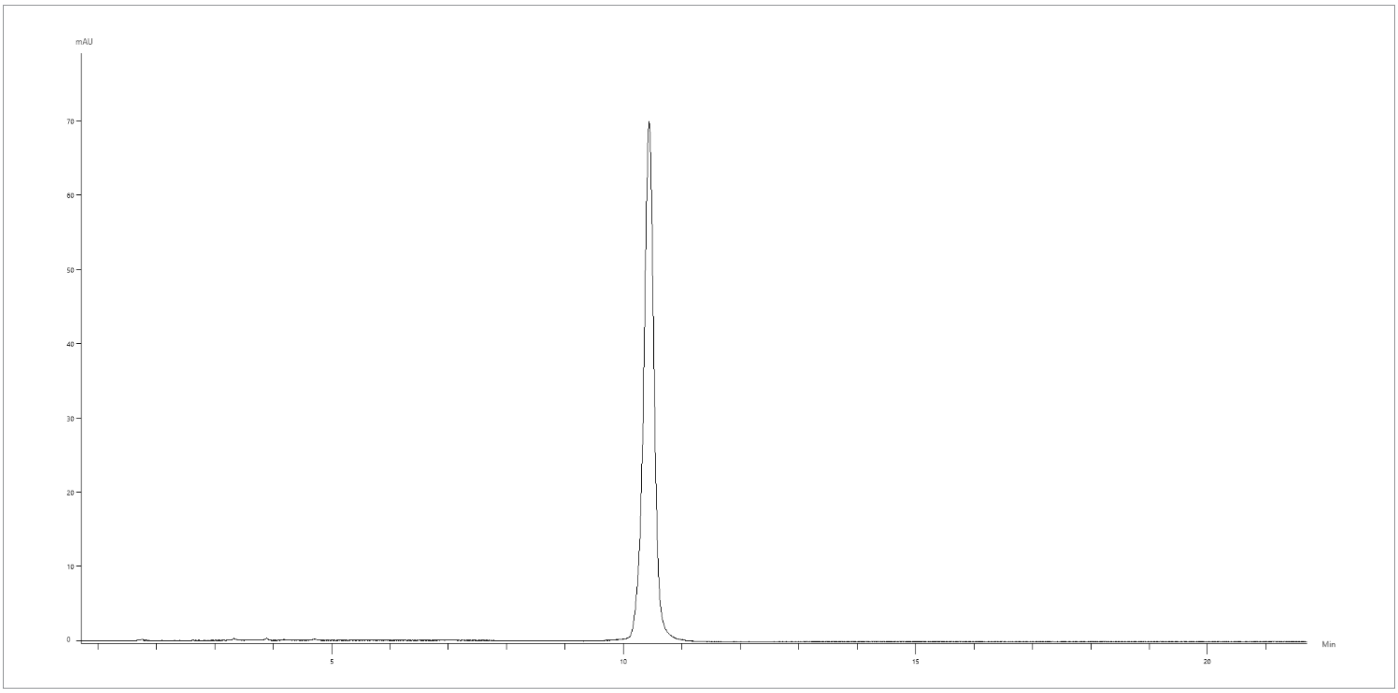

Figure 2: Assay analysis of Pregabalin using an Epic C18 column (250 x 4.6 mm, 5 µm).

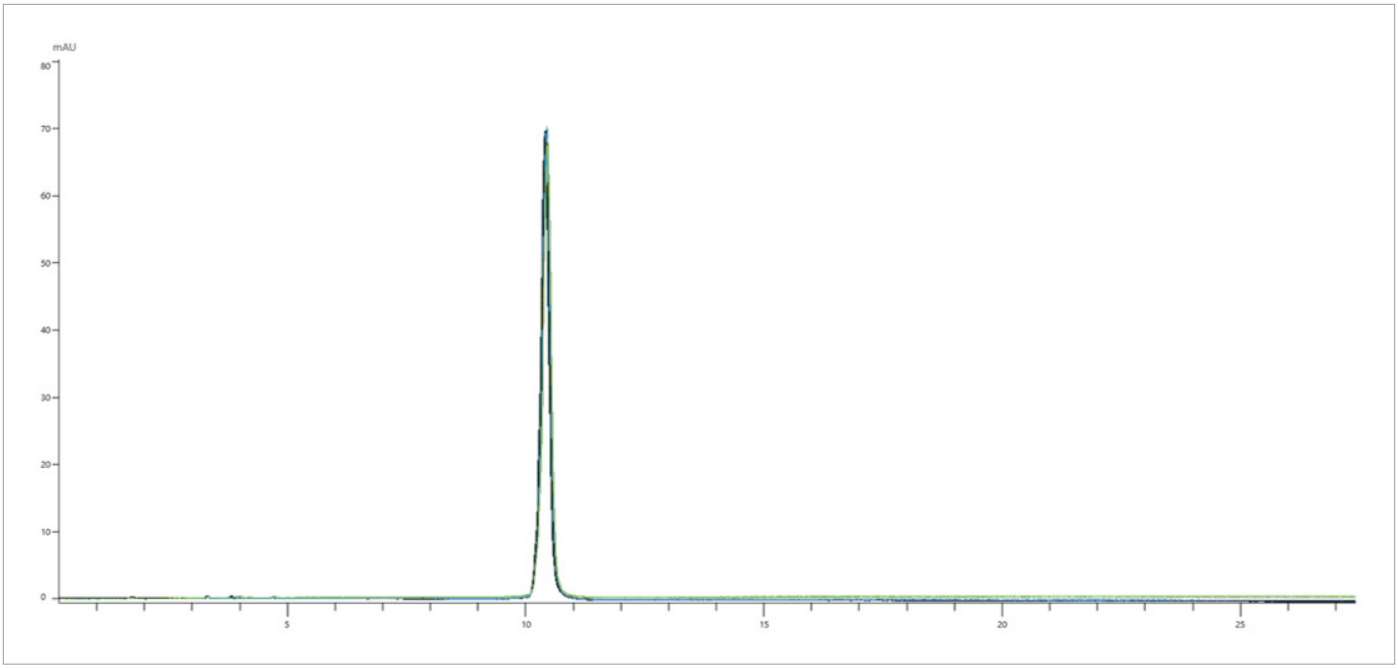

Figure 3: Overlay chromatogram.

Consumables

Name	Definition	Part Number
Column	Epic C18 column (250 x 4.6 mm, 5 µm)	155291-EC18
HPLC Vials	2 mL clear glass 10 mm screw top vial with write-on patch and fill lines, 100 pack	N9306053
Syringes	Syringe 1 mL BD Luer-Lok Disposable, Pack of 100	02542890
Syringe Filters	0.45 µm nylon filter, 13 mm diameter	N9306203
HPLC Vial Caps	Screw cap for 2 mL 10 mm vial with pre-split septa	N9306052
PEEK Fittings	Finger-tight for 1/16" OD PEEK tubing	09920513
Stainless Steel Fittings	OptiTech reusable nut/ferrule for UHPLC	N9306301

PerkinElmer, Inc.
940 Winter Street
Waltham, MA 02451 USA
P: (800) 762-4000 or
(+1) 203-925-4602
www.perkinelmer.com

For a complete listing of our global offices, visit www.perkinelmer.com/ContactUs